Role of plasma membrane nanostructures during heat sensing

(Final Report)

While the majority of the current cellular heat stress management studies focus on the roles of heat shock proteins (HSPs) and proteostasis network under severe stress conditions, the mild, fever-type stress and the maintenance of membrane homeostasis are noticeably less well researched. Dissection of membrane homeostasis is challenging as it requires multidisciplinary approaches that span cell biology, biophysics, biochemistry, and mass spectrometry-based lipidomics, to enable the identification of novel membrane sensors that control large-scale cellular thermal adaptation. The two laboratories collaborating in the current project aimed to identify heat induced specific alterations of membrane nanostructures leading to the generation of stress signals by combining our complement expertise on the field of molecular stress biology (Szeged) and single molecule microscopy (Vienna).

During the first year of the project we have successfully introduced and validated the ultrasensitive (TOCCSL and ImFCS) and high content (using Laurdan analogues) fluorescence microscopy techniques to study the structural changes occurring in the cellular plasma membrane during the perception of mild heat shock (HS). By using these tools together with standard molecular biology and biochemistry methods we have investigated the acute effect of heat on membrane organization. We have also analyzed the changes in cellular lipidomics occurring during the first hour of the heat treatment and introduced giant unilamellar vesicles to model the detected changes observed in the membrane lipid composition. We have published 6 original research paper, 1 review and 1 PhD thesis based partially or fully (Brameshuber et al., 2015; Peksel et al., 2017a, Peksel, 2017b) on the current project.

Based on their HSP response, we identified three distinct heat doses for CHO and MEF cells, namely: mild (40°C; eliciting a novel eustress without HSP induction), moderate (42.5°C), and severe (44°C). The induction of HSPs is a well-characterized stress response, but it happens hours after the perception of stress. Our study has validated rapid stress dose-dependent redistribution of HSPs as a reliable measure of the stress insult, much closer in time to its onset. Differences in the induction and cellular redistribution of HSPs suggest the operation of strikingly different sensing and signaling mechanisms during these different heat doses. While mild heat does not affect HSF1 phosphorylation and HSPs induction, it initiates the translocation of HSP25 and HSP70 to the nucleus. The nuclear localization of HSP25 is known to be linked to cytoprotection and was suggested to protect nucleic acids from DNA fragmentation induced by a severe heat shock (43°C, 3 h), thus preventing apoptotic cell death. Small HSPs, e.g., HSP25, are real molecular factotums with multiple moonlighting functions

during stress. Apart from their classical anti-apoptotic chaperone function, they can also interact with membranes, stabilizing the membrane structure and rescuing heat-denatured membrane-associated proteins. The role of nuclear translocation of HSP70 under mild, eustress conditions cannot be gleaned from previous studies in the lack of defined stress doses. Under moderate and severe HS, however, it stabilizes protein complexes in the cytoplasm and temporarily translocates to the nucleus. Inside the nucleus, it not only protects thermolabile proteins but also facilitates the storage of unfolded proteins within large insoluble foci.

The induction of thermotolerance at mild, fever-range temperatures, e.g., 39–40°C, has received little attention thus far. It is remarkable that the mild heat-induced changes reported in our studies lead to an appreciable acquired thermotolerance (ATT) in CHO cells. This suggests that the cells remember and are prepared for the next stress even without the contribution of newly synthesized HSPs.

We introduced the term “cellular eustress” to define the mild HS described above. The ability of cells to become more resistant to a second heat shock has previously been linked to HSP production, however, other reports indicated that HSP induction may not be the sole prerequisite for the development of ATT. In the current study, no correlation between the development of ATT at 40°C and HSP synthesis was observed.

It is well established that subtle changes in the physico-chemical properties of surface membranes are the most upstream events of sensing and signaling of mild, fever-type heat stress Török et al., 2014). Experimental data suggest that membrane thermosensing stems from a perturbation of physico-chemical structures of signaling platforms whose specific participants remain unknown. To identify specific stress membrane sensors, the sequence of membrane events during the perception of heat should be first dissected.

Heat exposure significantly increases the lateral diffusion of fluorescent membrane probes in the PM of CHO cells at moderate (42.5°C), but not mild (40°C), temperatures. While the effect of mild heat was successfully balanced by a microdomain rearrangement (i.e., increased domain confinement), moderate heat resulted in both an increased diffusion and reduced confinement of GPI-mGFP. The increased confinement of GPI-mGFP upon mild HS can be explained by the formation and stabilization of actin-mediated liquid-order lipid nanoclusters, especially in the light of the observed lipidomics changes.

To allow a comprehensive assessment of the rapid heat-induced changes in the cellular lipid composition of CHO cells, lipidomics datasets were analyzed by using the data-mining sPLS-DA method. This led to an excellent separation of the experiments into four non-overlapping clusters. The lipidomes of stressed CHO cells differed from the control; the extent

of differences gradually increased with temperature and heat treatment resulted in distinct CHO lipidomic fingerprints, in a dose-dependent manner.

The key features of the heat-elicited lipid remodeling, in general, comprised the accumulation of Cer, long chain FA-containing SL species, CL (and PG), PS, and the depletion of LPC. Our lipidomic data and other recorded cellular processes (activation of p38 MAPK, SAPK/JNK, and ERK1/2, HSF1 phosphorylation, membrane stabilization, mitochondrial biogenesis) suggest that stress-induced global lipid metabolic reprogramming is co-regulated with these adaptive cellular functions. They also highlight the notion that comprehensive lipidomics is a valuable tool complementing membrane biophysics, protein translocation, and stress response signaling data.

How are HS signaling pathways linked to cellular membranes under mild or moderate HS? The described physico-chemical changes in the membranes could lead to altered membrane tension and permeability; lipid and membrane protein rearrangement; changes in transmembrane potential; and the formation of lipid peroxides and lipid adducts (Kültz, 2004). The most likely candidate membrane sensors under these conditions are receptors or receptor networks. The structural changes of members of this putative stress-sensing receptor network could constitute both a rapid signal and an immediate feedback to control the effectiveness of cellular stress response.

The results of the current project clearly show that HSP induction is not critical under short-term mild stress conditions in CHO cells. From the physiological perspective, it seems prudent that the first wave of defense to an acute mild HS should not depend on such time-consuming processes as transcription and translation, but rather, should act primarily by immediately impacting preexisting proteins. Our data allow a clear differentiation of a discrete mild eustress, when the cells adapt to fever-type mild heat by maintaining membrane homeostasis, activating lipid remodeling, and redistributing chaperone proteins without inducing HSP synthesis. At higher temperatures, additional defensive mechanisms are activated, including classical HSP expression, which contribute to an extended stress memory and ATT. Further studies are in progress to test the general validity of the above findings in various mammalian systems.

Our studies fill an important gap in knowledge because it highlights the complexity of understanding the heat shock response - which has many ramifications at all levels - chemically, morphologically, and functionally, especially as the stress itself can vary from a "tickle" to a smashing hammer blow.

References:

Kültz, D. (2004). Molecular and Evolutionary Basis of the Cellular Stress Response. *Annual Review of Physiology*, *67*(1), 225–257.

Török, Z., Crul, T., Maresca, B., Schütz, G. J., Viana, F., Dindia, L., … Harwood, J. L. (2014). Plasma membranes as heat stress sensors: From lipid-controlled molecular switches to therapeutic applications. Biochimica et Biophysica Acta - Biomembranes, 1838(6), 1594–1618.

Publications based on the results of the current project:

Research articles:

1. Escribá, P.V., Busquets, X., Inokuchi, J., Balogh, G., Török, Z., Horváth, I., Harwood, J.L., Vígh, L. (2015) Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog. Lipid. Res. **59**, 38-53. doi: 10.1016/j.plipres.2015.04.003
2. Brameshuber, M., Sevcsik, E., Rossboth, B., Manner, C., Deigner, H-P., Peksel, B., Péter, M., Török, Z., Hermetter, A., (2016) Oxidized phospholipids inhibit the formation of cholesterol-dependent plasma membrane nanoplatforms. *Biophysical J.* **110**, 205-2013. doi: 10.1016/j.bpj.2015.11.018
3. Glatz, A., Pilbat, A-M., Németh, G.L., Vince-Kontár, K., Jósvay, K., Hunya, A., Udvardy, A., Gombos, I., Péter, M., Balogh, G., Horváth, I., Vígh, L., Török, Z. (2015) Involvement of small heat shock proteins, trehalose, and lipids in the thermal stress management in Schizosaccharomyces pombe. *Cell Stress Chaperones* **21**, 327-338. doi: 10.1007/s12192-015-0662-4
4. Koprivanacz, K., Tőke, O., Besztercei, B., Juhász, T., Radnai, L., Merő, B., Mihály, J., Péter, M-, Balogh, G., Vígh, L., Buday, L., Liliom, K. (2017) The SH3 domain of Caskin1 binds to lysophosphatidic acid suggesting a direct role for the lipid in intracellular signaling. Cellular Signalling **32**, 66-75. doi: 10.1016/j.cellsig.2017.01.019
5. Péter, M., Glatz, A., Gudmann, P., Gombos, I., Török, Z., Horváth, I., Vígh, L., Balogh, G. (2017) Metabolic crosstalk between membrane and storage lipids facilitates heat stress management in *Schizosaccharomyces pombe. PLoS One*, **12**, e0173739. doi: 10.1371/journal.pone.0173739
6. Tóth, E.A., Oszvald, Á., Péter, M., Balogh, G., Osteikoetxea-Molnár, A., Bozó, M., Szabó-Meleg, E., Nyitrai, M., Derényi, I., Kellermayer, M., Yamaji, T., Hanada, K.,

Vígh, L., Matkó, J. (2017) Nanotubes connecting B lymphocytes: High impact of differentiation-dependent lipid composition on their growth and mechanics. Biochim. Biophys. Acta – Mol. Cell Biol. Lipids **1862**, 991–1000. doi: 10.1016/j.bbalip.2017.06.011

7. Peksel, B., Gombos, I., Péter, M., Vigh, L. Jr., Tiszlavicz, Á., Brameshuber, M., Balogh, G., Schütz, G.J., Horváth, I., Vígh, L., Török, Z. (2017a) Mild heat induces a distinct "eustress" response in Chinese Hamster Ovary cells but does not induce heat shock protein synthesis". *Scientific Reports* 7: 15643. doi:10.1038/s41598-017-15821-8

Phd thesis:

Begüm Peksel Şahin (2017b) Sensing mechanisms and individuality of heat stress in mammalian cells (supervisor: Zsolt Török)