Summary of the PhD thesis

Sensing mechanisms and individuality of heat stress in mammalian cells

Begüm PEKSEL ŞAHİN

Supervisor: Dr. Zsolt TÖRÖK

Doctoral School of Environmental Sciences

University of Szeged

Institute of Biochemistry

Biological Research Center of the Hungarian Academy of Sciences

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INTRODUCTION

Stress response is a vitally important biological process that protects organisms against various environmental and pathophysiological conditions. One of the most studied stress is heat that results in deleterious damages, i.e. leakage of the plasma membrane or severe protein denaturation, only at relatively high temperatures. However, heat shock response could also be activated under mild pathophysiological conditions, such as in fever, that is unlikely to cause proteotoxicity. Recently, considerable evidence has been accumulated in favor of the "Membrane sensor hypothesis" which predicts that, the level of heat shock proteins (HSPs, also called stress proteins) changes as a result of alterations to the plasma membrane. Stressful conditions activate numerous membrane-related sensors at the top of the signalling pathways which are interconnected by the parameters of the membrane chemical and physical state. Phospholipids, sphingolipids and cholesterol are all involved in the generation of stress-induced second messengers.

The study of cellular stress response is of great importance to our understanding of how cells respond and adapt to various changes in their environments. Therefore, a systematic approach is needed to investigate the sequence of heat-induced cellular events as a function of exposure time and temperature to better understand the roles of HSP synthesis, HSP redistribution, re-modelling of plasma membrane structure and cellular lipidome, as well as the possible signal transduction pathways in stress response.

Herein, our current work characterized the acute effect of mild heat shock on membrane organization, and HSP synthesis and localization in mammalian cells, to delineate the role of membranes in the sensing and adaptation to heat by combining ultrasensitive fluorescence microscopy and lipidomics.

Another fundamental question is whether all cells in a culture (or in tissue) suffer the same consequence of an imposed stress and whether they respond in the same manner. Knowledge on gene expression and cellular responses in cells is derived from analyses of populations consisting of millions of cells. Although this approach provides useful insights into average population responses, it does not provide information on individual cells or subpopulations within cell cultures. The impact of heterogeneity is significant in many aspects. First, sample heterogeneity is critical for the functionality of cellular networks. Second, cell ensemble data can easily be misinterpreted when ignoring heterogeneity; a specific parameter – e.g. an expression profile, the molecular clustering or mobility – determined by measuring the average ensemble value need not to be preserved in the individual subclasses of cells. Third, correlation analysis at the single cell level allows for identifying

molecular implications in a systemic approach; in this aspect, sample heterogeneity is utilized for obtaining mechanistic insights into cellular processes.

In the present thesis we demonstrated the individual variability in the stress response of a genetically homogeneous mammalian cell population. Cell cycle phase, as a first cause of heterogeneity, is linked to differential stress responses. However, methods such as serum starvation and chemical treatments to block the cell cycle have collateral effects on the lipid composition of the cells. Therefore, results obtained with such methods can be misleading. The combination of microscopy and fluorescence-activated cell sorting - applied in this study - allows the analysis of the cell cycle phases and the individuality of stress response without chemical treatments.

AIMS OF THE STUDY

The major aim of this study was to understand how cells sense the heat, how membranes contribute to the heat sensing and cellular response to heat. The questions addressed are as follows:

- What is the sequence of early events during the perception of temperature stress?
- How different temperature treatments (heat dose) affect the cellular response in terms of membrane-dependent changes, protein induction and activation of stress signalling pathways?
- Does the altered membrane structure and composition relate with the heat resistance?
- How cell cycle affects the stress response and to what extent it contributes to it?
- What are the early events of heat sensing? Is it possible to use these as parameters to analyze the individuality of the stress response?
- Is the population heterogeneity of the heat stress signal linked to the membrane?

METHODS

Cell culture
Western blot
Colony formation assay
Immunofluorescence labelling and Fluorescence microscopy
High-throughput microscopy and image analysis
Total internal reflection fluorescence microscopy (TIRF)
Image-based Fluorescence Correlation Spectroscopy (ImFCS)
Thinning out clusters while conserving stoichiometry of labeling (TOCCSL) experiments

Plasma membrane isolation and plasma membrane sheet preparation

Lipidomic analysis by electrospray mass spectrometry (ESI-MS)

Measurement of intracellular calcium levels

Fluorescence-activated cell sorting (FACS)

SUMMARY OF FINDINGS

The most important conclusions that can be derived from this work can be listed as follows:

- Early events during the perception of temperature stress involve the remodelling of the plasma membrane and cellular lipidome, hyperphosphorylation of Heat shock factor 1(HSF1), activation of MAPK signalling pathways and redistribution of HSPs that is followed by an increase in HSP protein levels and gain of acquired thermotolerance.
- Cellular response of CHO cells to heat is both temperature- and duration-dependent.
- Distinct heat stress levels, namely mild (40°C), moderate (42.5°C) and severe (44°C) can be classified based on distinct cellular lipidomes, plasma membrane organizations, HSP response and signalling pathways.
- Mild stress induces ERK1/2 signalling whereas JNK and p38 MAPK pathways are more active at higher temperatures
- Cells promptly respond to heat by remodelling their plasma membrane. Heat exposure significantly increases the lateral diffusion of fluorescent membrane probes in the plasma membrane of CHO cells at moderate, but not at mild temperatures. While the effect of mild heat is successfully balanced by an active microdomain rearrangement (i.e., increased domain confinement), moderate heat resulted in both an increased diffusion and reduced confinement of GPI-mGFP.
- Cells instantly respond to heat by remodelling their lipidome. Phosphatidylserine, phosphatidylglycerol, cardiolipin, lysophosphatidylcholine and ceramide changes have been identified as general key features, while the alteration in phosphatidic acid is distinctive for moderate and severe stress.
- Re-distribution of HSP25 and HSP70 into nuclear and perinuclear compartments is an acute effect of heat and reflects dose-dependent stress response.
- HSP25 and HSP70 protein levels are controlled with a different threshold or possibly through different mechanisms that are not connected to HSF1 phosphorylation on Ser326.

- Our results delineated the molecular details of a novel mild type of cellular eustress, when the mammalian cells adapt to fever-type mild heat by maintaining membrane homeostasis, activating lipid remodelling, and redistributing chaperone proteins leading to acquired thermotolerance; strikingly, these processes occur in the complete absence of the induction of HSPs. At higher temperatures, additional defense mechanisms are activated, including elevated expression of molecular chaperones contributing to an extended stress memory and acquired thermotolerance.
- At mild temperatures, heat treatment alters lipid composition and plasma membrane structure and it results in a significant increase of acquired thermotolerance, therefore pointing out a possible interrelationship of the two.
- Individual variation of the cellular heat shock response is observed in translocation of HSPs to the nucleus (early response) and in induction of HSPs (late response).
- Cell-to-cell variation of heat-induced HSP25 redistribution is a cell cycle-independent event whereas distinct lipid profiles of G1, S and G2 phases are observed.
- Single cell analysis of heat-induced Ca^{2+} signalling indicates that the population heterogeneity of the heat stress signalling can be linked to membranes, but further studies are required in order to better understand this phenomenon.

PUBLICATIONS

Publications that are related to the PhD thesis:

Peksel, Begüm, Imre Gombos, Mária Péter, László Vigh, Jr., Ádám Tiszlavicz, Mario Brameshuber, Gábor Balogh, Gerhard Schütz, Ibolya Horváth, László Vígh, Zsolt Török. "**Mild heat induces a distinct "eustress" response Chinese Hamster Ovary cells that does not require heat shock protein synthesis**". (2017) Scientific Reports (in press)

I.F.: 4.259

Torok, Zsolt, Tim Crul, Bruno Maresca, Gerhard J Schutz, Felix Viana, Laura Dindia, Stefano Piotto, Mario Brameshuber, Gabor Balogh, Maria Peter, Amalia Porta, Alfonso Trapani, Imre Gombos, Attila Glatz, Burcin Gungor, Begum Peksel, Laszlo Jr Vigh, Balint Csoboz, Ibolya Horvath, Mathilakath M. Vijayan, Phillip L. Hooper, John L. Harwood, Laszlo Vigh. "**Plasma Membranes as Heat Stress Sensors: From Lipid-Controlled Molecular Switches to Therapeutic Applications**." Biochimica et Biophysica Acta 1838, no. 6 (June 2014): 1594–1618. doi:10.1016/j.bbamem.2013.12.015.

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I.F.: 3.656

Publications that are not directly related to the PhD thesis:

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I.F.: 5.547

Molnár, Eszter, Soujanya Kuntam, Pradeep Kumar Reddy Cingaram, Begüm Peksel, Bhavyashree Suresh, Gabriella Fábián, Liliána Z. Fehér, Attila Bokros, Agnes Medgyesi, Ferhan Ayaydin, Laszlo G. Puskas. "**Combination of Small Molecule Microarray and Confocal Microscopy Techniques for Live Cell Staining Fluorescent Dye Discovery.**" Molecules 18, no. 8 (August 2013): 9999–10013.

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I.F.: 0.28