PhD thesis

Membrane regulated stress response in mammalian cells

Presented by Enikő Éva Nagy
Supervisor: Prof. László Vígh

Biology PhD School
University of Szeged

Hungarian Academy of Sciences
Biological Research Centre Institute of Biochemistry
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INTRODUCTION

The ability of living organisms to cope with the constantly changing environmental conditions is essential for permanently maintaining life on Earth. Abrupt, temporal environmental changes induce a prompt and transient reaction called the stress response. Cellular stress response is a universal mechanism of extraordinary physiological and pathological importance. It represents a defence reaction of cells to damage that environmental forces inflict on macromolecules. The stress response is accompanied with characteristic changes in the gene expression pattern inducing the accumulation of proteins with protective function. One group of these proteins are the highly conserved heat shock proteins (Hsps) which are activated or induced during many types of stress.

Hsps contribute to the maintenance of protein homeostasis in various ways. They are molecular chaperons that bind and stabilize proteins with instable conformation and assist in proper folding of misfolded proteins to achieve their native forms. Our research group has shown that a subpopulation of Hsps is present either on the surface or within the cellular membranes. Via their specific protein and lipid interactions Hsps can control major attributes of the membrane like fluidity, permeability or non-bilayer propensity. Hsps play a fundamental role in the pathology of several human diseases. Aberrantly high level of certain Hsp classes is characteristic in cancer cells and the converse situation applies for type 2 diabetes or

neurodegeneration. In accordance, understanding the mechanism whereby cells can elicit a stress protein response is of key importance.

In spite of the knowledge on the function and expressional regulation of Hsps, basic questions on the molecular mechanism of stress perception, the identity of primary stress sensors are still remained open.

Our concept that membranes are potential stress sensors arise from studies performed mainly in prokaryotic organisms. The membrane sensor hypothesis postulates that membranes are not only the targets of cellular damage during stress but alterations of membrane physical state and/or composition may function in initial stress sensing events and in adaptation processes. In the proposed model the crosstalk between membranes and *hsp* gene expression forms a feedback regulatory loop in the following way. During stress conditions modification of membrane physical state, membrane hyperfluidization activates the transcription of *hsp* genes. The Hsps synthesized besides protecting/refolding (mis)folded proteins interact with membranes thus re-establish the proper membrane lipid order and physical state. This in turn switches off stress signal generation in membranes leading to diminution of stress response.

AIMS OF THE STUDY

Our aim was to explore the possible involvement of mammalian cell membranes in stress sensing mechanisms. **The main goals** of the study were

1) To test the validity of "membrane sensor hypothesis" in mammalian cells

2) To characterize membrane alterations casually linked to stress response initiation

3) To identify signal transduction events effected by membranes during the initial phase of stress response

4) To analyze the role of heat shock factor 1 in the membrane originated stress response

5) To test the potential of membrane perturbation in conferring resistance for cells to a subsequent severe stress

METHODS

Cell culturing and transient transfection

In vivo protein labelling, Western blotting

Quantitative polymerase chain reaction

Electrophoretic mobility shift assay

Chromatin immunoprecipitation

Reporter activity measurement

Membrane fluidity measurement

Steady state fluorescence quenching measurement

Cholesterol rich plasma membrane domain labelling by fluorescent probe and confocal microscopy

Measurement of intracellular free Ca^{2+} concentration

Clonogenic survival assay for testing thermotolerance

RESULTS

1. To unravel the validity of "membrane sensor hypothesis" in mammalian cells we asked that changes in the physical state of membranes could influence the stress response. The physical state of membranes was modified by the administration of a well characterised membrane intercalating agent namely benzyl-alcohol (BA) then its effect on the activation of the stress response was followed. We demonstrated that the expression of *hsp* genes was induced by BA treatment even at growth temperature in several mammalian cell lines.

2. In the widely accepted model the protein denaturation is considered as the primary stress-sensor initiating the stress protein response. Following the activity of heterologously expressed firefly luciferase reporter enzyme we found that BA exerts no measurable effect on protein denaturation.

Next we focused on membrane organization changes induced by stress and tended to reveal alterations which can be causally related to Hsp response initiation.

3. First it was proposed that a critical drop in the membrane physical order can generate sufficient signal for *hsp* gene induction. To test this assumption the effects of another, structurally similar membrane fluidizer molecule (phenethyl-alcohol) were studied. We provided evidence, that phenethyl-alcohol at a concentration equipotent with BA in membrane fluidization, does not generate a stress protein signal.

4. To unravel some possible mechanisms underlying stress response initiation besides membrane hyperfluidization we examined the lateral organization of membranes. We demonstrated that treatments inducing Hsp response (like BA and heat) also have the potential to destabilize cholesterol-rich ordered lipid domains *in vitro* and to reorganize specific cholesterol-rich plasma membrane microdomains *in vivo*.

We were also interested in the signal transduction events affected by membrane alterations and leading to the induction of *hsp* genes.

5. The role of Ca^{2+} was suggested, since it has been known that Ca^{2+} is a key signalling element in the heat stress response pathway. In our experiments an increase in the intracellular free Ca^{2+} concentration was detected upon BA treatment. This rise in Ca^{2+} level proved to be necessary for efficient induction of *hsp70*.

6. Heat shock factor 1 is the main transcriptional regulator of *hsp* induction under stress conditions. We provided evidence that similar to heat treatment, HSF1 undergoes its multistep activation process upon BA administration: it acquires DNA binding ability, becomes

hyperphosphorylated and binds to the *hsp70* promoter in the cells. It was also verified that this activation is a necessary step for the induction of *hsp* genes.

Stress triggers a complex response which, besides promoting the restoration of damaged macromolecular functions, supports also cells to survive under persisting stressful conditions. This phenomenon is called acquired stress tolerance.

7. Accordingly, finally we addressed the important question whether stress signals originated from membranes can also induce processes rendering cells resistant to subsequent lethal stress. Testing the thermal sensitivity of cells by their colony forming ability we confirmed that similar to sublethal temperature pretreatment, preconditioning by the administration of BA is fully sufficient for the development of cellular thermotolerance.

In conclusion, our results strongly indicate that in addition to proteins, DNA and redox state, the membranes of mammalian cells play a critical role in thermal sensing as well as signalling. The exact mechanism of membrane mediated stress perception imposed by BA awaits further studies but membrane hyperfluidization and the distinct re-organization of cholesterol-rich microdomains are also required for the generation of stress signal. The rise of intracellular Ca^{2+} and the activation of HSF1 are already identified elements of the signal transduction pathway leading to the synthesis of Hsps. Similar to heat priming, isothermal membrane perturbation can also trigger the

general stress response processes protecting cells from subsequent heat damage.

THESIS POINTS

1. Benzyl alcohol provoked membrane perturbation induces the expression of *hsp* genes even at growth temperature.
2. Administration of benzyl alcohol exerts no measurable effect on protein denaturation.
3. Membrane hyperfluidization is not necessarily coupled with the induction of heat shock genes.
4. The Hsp response inducer benzyl alcohol and heat stress apart from membrane hyperfluidization also have the potential to re-organize specific cholesterol-rich plasma membrane microdomains.
5. Administration of benzyl alcohol increases intracellular free Ca^{2+} level, which is necessary for the induction of *hsp* genes.
6. Heat shock factor 1 undergoes its multistep activation process upon benzyl alcohol treatment.
7. Preconditioning by the administration of benzyl alcohol is fully sufficient to confer cellular thermotolerance.

PUBLICATONS RELATED TO THE THESIS:

Nagy E, Balogi Z, Gombos I, Akerfelt M, Bjorkbom A, Balogh G, Torok Z, Maslyanko A, Fiszer-Kierzkowska A, Lisowska K, Slotte PJ, Sistonen L, Horvath I, Vigh L.: Hyperfluidization-coupled membrane microdomain reorganization is linked to activation of the heat shock response in a murine melanoma cell line. *Proc. Natl. Acad. Sci.* (2007) 104(19) 7945-50.

Balogh G, Horváth I, **Nagy E**, Hoyk Z, Benkő S, Bensaude O, Vígh L.: The hyperfluidization of mammalian cell membranes acts as a signal to initiate the heat shock response. *FEBS J.* (2005) 272(23) 6077-86.

OTHER PUBLICATIONS:

Sőti C, **Nagy E**, Giricz Z, Vígh L, Csermely P, Ferdinandy P.: Heat shock proteins as emerging therapeutic targets. *British J. Pharmacol.* (2005) 146(6) 769-80.

Nagy L, Csintalan G, Kalman E, **Nagy E,** Sipos P.: Application of metal ions and their complexes in medicine II. Application of platina complexes in the treatment of tumor. *Acta Pharm. Hung.* (2004) 74(4) 213-22, Hungarian.

Török Z, Tsvetkova NM, Balogh G, Horváth I, **Nagy E,** Pénzes Z, Hargitai J, Bensaude O, Csermely P, Crowe JH, Maresca B, Vígh L.: Heat shock protein coinducers with no effect on protein denaturation specifically modulate the membrane lipid phase. *Proc. Natl. Acad. Sci.* (2003) 100(6) 3131-36.

Nagy L, Gubany E, Pellerito C, **Nagy E,** Szorcsik A.: Biological and chemical properties of alkyl lead derivatives. *Acta Pharm. Hung.* (2003) 73(2) 103-14, Hungarian.