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DISSERTATION SUMMARY

Connection between membrane physical state and heat shock response

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The classical model on the sensing/signaling of heat shock proposes that primarily the accumulation of denatured proteins triggers the activation of the stress-response. Our alternative, but not exclusive, view is that the mechanism of temperature sensing is associated also with the alterations of the physical state of membranes (Horváth et al. 1998; Vígh et al. 1998; Vígh and Maresca 2002).

In our recent study the membrane fluidizing agent benzyl alcohol (BA) was used to test the role of proteootoxicity and membrane perturbation separately, in generation of HS response (Balogh et al. 2002). Mammalian cells treated with BA at their growth temperature showed an elevated synthesis of the major stress protein, HSP70. By monitoring the enzymatic activity of luciferase expressed in mammalian cells we have demonstrated that unlike heat stress, BA treatment does not result in protein denaturation. Furthermore, it was also evidenced that the non-toxic cytoprotective drug candidate bimoclomol, which is a coinducer of HSPs, does not affect protein denaturation *in vivo*. Bimoclomol, however, interacts specifically with and increases significantly the fluidity of negatively charged membrane lipids even at normal temperatures (Török et al. 2003). Based on these results we can hypothesize that there are signaling cascades at least with two origins leading to the expression of heat shock genes. One is linked to the formation of denaturated proteins and the other is derived from the lipid phase changes of the membranes. In order to further understanding the mostly unknown regulatory mechanisms acting in response to different stressors, an extensive structural and functional analysis of the promoter region of the hsp70i gene was performed by using B16 melanoma cells. Series of plasmids containing the bacterial chloramphenicol acetyltransferase (CAT) as a reporter gene under the control of different fragments of the rat hsp70.1 promoter were used: namely, each of three HSEs alone, combination of two HSEs, all three HSEs or only proximal promoter sequences without any HSE. These promoter regions directed variable expression of the reporter, however, the induction pattern was similar by mild heat or BA treatment. Our data further supports the hypothesis that subtle alterations of membrane physical state is involved in the conversion of heat stress into sequential processes culminating in the transcriptional activation of stress regulated genes. In order to reveal and analyse the hypothetical heat sensing elements and the connected signalling pathways, different metabolic inhibitors have been introduced. Our preliminary results on the role of intracellular Ca^{2+} and the possible involvement of p38 MAP kinase will be discussed.

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