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Methods 35 (2005) 139–148
METHODS
www.elsevier.com/locate/ymeth

Regulatory interfaces between the stress protein response and other gene expression programs in the cell

Stuart K. Calderwood*

Division of Molecular and Cellular Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, 21–27 Burlington Avenue, Boston, MA 02215, USA

Accepted 20 August 2004
Available online 19 December 2004

Abstract

The stress protein response involves the immediate reprogramming of gene expression in cells exposed to proteomic insult leading to massive synthesis of heat shock proteins (HSP). We have examined the outcome when cells are induced to activate two other gene expression programs—the acute inflammatory response and entry of quiescent cells into the cell cycle—and then exposed to protein stress. We find that these responses are mutually antagonistic with, on the one hand, heat shock factor 1 (HSF1) inhibition through the phosphorylation of inhibitory serine residues after inflammatory or mitogenic stimulus and, on the other hand, after stress, HSF1 directly repressing the promoters of genes that mediate acute inflammation and mitogenesis. The expression of the stress protein response during periods of acute protein damage was shown to lead to efficient activation of HSF1 and HSP expression accompanied by repression of other gene expression programs.

Keywords: Heat shock protein expression; Heat shock factor; Activation; Repression; Inflammation; Mitogenesis

1. Introduction

The stress protein response is a remarkably potent mechanism for resisting stress that can convert a cell population normally killed down to one cell in 10,000 by protein stress into a population in which most cells can survive [1]. It is particularly intriguing in that many of the proteins that mediate the response are conserved throughout cellular history [2,3]. The effector proteins of the response, heat shock proteins (HSP)[1] are remarkably conserved in *archaea*, prokaryotes and eukaryotes [2,3]. The regulatory proteins that control HSP transcription, heat shock factor (HSF) are likewise conserved in the eukaryotes [4]. This led to the notion that the heat shock response behaves as a "molecular fossil" controlled in isolation from the surrounding regulatory networks and functioning independently as an autonomous sentinel guarding the cellular proteome. This notion is, however, dissipating as evidence increases indicating that the stress protein response is integrated into cell and tissue-wide regulatory matrices.

2. Regulatory intermediates of the stress protein response in mammalian cells

We will first briefly consider the regulation of HSF1 during protein stress as this has major implications for cross talk with other systems. In mammalian cells, a cohort of molecular chaperone proteins is induced by stress, including "small HSP" typified by HSP27 as well as HSP40, HSP60, HSP70, HSP90, and HSP110 [2,3]. Some of the proteins, the HSP70 family in particular,

* Fax: +1 617 632 0635.
E-mail address: scalderw@bidmc.harvard.edu.

[1] *Abbreviations used:* HSP, heat shock protein; HSF, heat shock factor; IL1β, interleukin 1β; NF-IL6, nuclear factor of interleukin 6; NFκB, nuclear factor κB; ERK, extracellular signal regulated protein kinase; GSK3, glycogen synthase kinase 3.

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doi:10.1016/j.ymeth.2004.08.004

are encoded by more than one gene. Mammalian cells contain three HSF family members including HSF1, HSF2 and HSF4 [4–6]. No gene corresponding to avian *hsf3* has so far been observed [7]. Transcriptional regulation during protein stress involves mainly HSF1, with HSF2 and HSF4 carrying out auxiliary or tissue specific roles [6,8].

Transcriptional activation involves release from intramolecular associations that mask the trimerization domains permitting trimerization, nuclear localization, and binding to the heat shock elements in HSP promoters [9–11]. These events involve a considerable unfolding of HSF1 reflected in the hydrodynamic properties of HSF1 [9,10], and this unfolding appears to be necessary for interactions with other molecules such as protein kinases and transcription factors that mediate cross talk with other responses in the cell [12,13]. For full transcriptional activation, a further stress-induced step is required that is dependent on the activities of upstream tyrosine kinases and results in the hyperphosphorylation of HSF1 largely on serine residues [14–16]. The primary regulator of HSF1 in the cell is one of its products, HSP90. HSP90 maintains HSF1 in its inactive, compacted form in the cell [17]. The central role of HSP90 in this regard is indicated by that fact that HSP90 inhibitors such as geldanomycin can activate all steps of the stress protein response [17]. HSF1 is an unusual HSP90 client protein in that, while other HSP90-associated proteins become destabilized and destroyed by proteolysis following HSP90 dissociation, HSF1 is activated and leads to abundant HSP expression [17]. HSF1 appears to be repressed by a number of other pathways largely mediated by phosphorylation. These include repression mediated by a double phosphorylation at serines 307 and 303 by the ERK and GSK3 pathways, and by phosphorylation at serine 363 by protein kinase C [18–21].

3. Stress protein response in physiology and pathology

Stress proteins play a number of roles in cell and tissue physiology. As mentioned, HSP protect the proteome through their molecular chaperone function that permits them to recognize damaged proteins and either channel such proteins into repair/ refolding pathways or to proteolysis. In terms of cell survival, this permits cells to respond to damage at source and immediately begin the processes required to resolve the cellular insult [22,23]. In addition, HSP may play a more generic role in cell survival and have been implicated as inhibitors of a remarkable number of steps in apoptosis, including both intrinsic and extrinsic pathways (reviewed in [24]). HSP may thus have been hijacked from their ancient role to play a part in other processes that require cells to negotiate stressful periods. In this regard, the molecular chaperone properties of HSP, particularly of the 70 and 90 kDa families, appear to have led the proteins to play a role in cell regulation, often as stabilizing inhibitory components of transcription factor or protein kinase complexes [24]. Another feature of the stress protein response is the power of the gene expression system involved and the high abundance of HSP expression in stressed cells [4]. This appears to have led to a further elaboration of the functions of stress proteins in the immune system [25,26]. Dying cells often undergo the stress protein response, leading to lysis and release of HSP into the extracellular space [27]. Such extracellular HSP appears to lead to a danger response that can activate the inflammatory response as well as the innate and adaptive immune response [25,28,29]. The pro-immune effects of extracellular HSP appear to be countered by the intracellular stress protein response and both HSF1 and the HSP70 are able to inhibit the expression of proinflammatory cytokines and dampen the potentially toxic effects of over-stimulation of the acute phase response [30,31].

Dysregulation of the stress protein response may play a role in pathology in a range of organs. Aging is associated with the degeneration of HSP expression with time and the loss of resistance to cellular oxidants: elevated HSF1 and HSP levels lead to significant increases in lifespan in a number of model systems including *Caenorhabditis elegans* and *Drosophila* [32–37]. Many of the underlying processes in neurodegeneration are associated with the decreased expression of molecular chaperones with time and the accumulation of tangled and aggregated proteins which are toxic to neurones [38–40]. In cancer the converse situation applies and malignant transformation is associated with aberrantly high levels of HSF1 and HSP [41–44]. Elevated HSP levels may lead to transformation partially through decreased cell death and upsetting of the equilibrium between cell birth and death rates [45,46]. The etiology of these disease processes reflect what is known of the functions of HSP molecular chaperones in cellular regulation, which indicate that either up or down regulation of HSP expression can drastically modulate multiple key proteins within the cell [24]. Understanding the stress protein response and its place in regulation in the cell and whole organism is therefore of key importance in the study of a range of human diseases.

3.1. Interactions between the stress protein response and immune and inflammatory responses

Suspicions that there may be a link between the heat shock and immune response date back many years. In fact, an early treatment for cancer involved the use of bacterial toxins, potent activators of cytokine cascades that lead to a rise in body temperature thought to be involved in tumor regression [47,48]. One of the key