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Stressing Rac, Ras, and Downstream Heat Shock Protein 70

Karin E. Bornfeldt

Stress proteins (also known as heat shock proteins) regulate fundamental cellular processes, such as protein folding, protein sorting, protein degradation, assembly of proteins into larger complexes, and resolubilization of aggregates.¹⁻⁴ Heat shock proteins (hsps) are divided into 6 subfamilies based on molecular mass: large hsps (110 to 100 kDa), hsp90, hsp70, hsp60, hsp40, and small hsps (30 to 18 kDa).⁵ Members of the hsp70 group are abundant in eukaryotic cells, and most research to date has focused on stress-inducible hsp70. hsp70 is induced by a number of stress stimuli, and its expression is regulated at the transcriptional level by heat shock transcription factor 1 (HSF1). HSF1, which normally exists as a monomer, trimerizes after activation and binds to a specific DNA recognition sequence (heat shock element) in the hsp70 promoter. HSF1 seems to require phosphorylation for full activity; however, the identity of the putative HSF1-activating kinase remains to be elucidated. It has been shown that HSF1 can be phosphorylated by extracellular signal-regulated protein kinases (ERKs) of the mitogen-activated protein kinase (MAPK) family in a Ras-dependent manner; however, this represses rather than stimulates transcriptional activity.⁶⁻⁸ It is also possible that HSF1 is activated by mechanisms that do not rely on phosphorylation, or that dephosphorylation of inhibitory phosphorylation sites stimulates the transcriptional activity of HSF1.

Although the first evidence of existence of hsps dates back to the early 1960s,⁹ hsps have recently received substantial attention in the field of cardiovascular research after several important and interesting observations. hsp70 has been shown to protect cells from apoptosis and necrosis induced by various stimuli, induce cross-tolerance of stressed cells exposed to a different deleterious stimulus,¹⁰ and protect cardiomyocytes from ischemia in vitro and in vivo.¹ Furthermore, induction of hsp70 has been demonstrated in the rat arterial wall subjected to acute hypertension,¹¹ human arteries subjected to balloon angioplasty,¹² and advanced lesions of atherosclerosis.¹³ Expression of another hsp, hsp47, has also recently been shown to be induced in the fibrous cap of human lesions of atherosclerosis, where it may regulate collagen secretion and plaque stability.¹⁴

Mechanical Forces Induce hsp70 Expression in Smooth Muscle Cells

In this issue of *Circulation Research*, Xu et al¹⁵ report that mechanical forces (cyclic strain stress) activate HSF1 and lead to subsequent hsp70 accumulation in cultured rat arterial smooth muscle cells (SMCs). This study is one in a series by this group on the effects of mechanical forces on SMCs. The finding that mechanical forces can increase hsp70 expression in cells is not novel per se. For example, hydrostatic pressure induces accumulation of hsp70 in human chondrocytic cells,¹⁶ and stretch upregulates hsp70 expression in rabbit hearts.¹⁷ What makes the study by Xu et al interesting is the finding that the small G protein Rac, and to a lesser extent Ras, is required for HSF1 activation and hsp70 expression. This observation is based on overexpression studies using dominant-inhibitory mutants of Rac and Ras, in which Ser17 has been mutated to an Asn. Xu et al additionally show that although ERK, c-Jun NH₂-terminal protein kinase/stress-activated protein kinase (JNK/SAPK), and p38 MAPK are all activated after mechanical stress in these cells, inhibition of ERK or p38 MAPK using chemical inhibitors does not block HSF1-DNA binding. Thus, Rac and Ras seem to be required for cyclic strain stress-induced HSF1 activation in a manner independent of ERK and p38 MAPK.

What Signaling Pathways Lead to hsp70 Induction After Cyclic Strain Stress?

What downstream signaling pathway may be responsible for the Rac- and Ras-induced hsp70 transcription in SMCs? G proteins (or GTP-binding proteins) are active when bound to GTP and inactive when bound to GDP. Activation of G proteins is induced by guanine-nucleotide exchange factors (GEFs) that accelerate release of GDP from the G proteins. The active GTP-bound G proteins then activate several downstream target enzymes (effectors). Ras has 3 major effectors, namely, the Raf serine and threonine protein kinases that act upstream of ERKs, lipid and protein kinase phosphatidylinositol 3-kinase (PI3K), and Ral-GDS, a GEF for the small G protein Ral.^{18,19} Because ERK is unlikely to activate HSF1,^{7,15} a probable mediator of cyclic strain stress-induced hsp70 expression seems to be PI3K. PI3K can be directly activated by Ras,²⁰ and the dominant-inhibitory Ras (N17) mutant inhibits PI3K activity.²¹ Furthermore, other studies have shown that Rac can be activated by a PI3K-dependent pathway.²² Thus, Ras can activate Rac through PI3K. Rac, in turn, activates the p21-activated protein kinases (PAKs), which are known to induce JNK/SAPK and p38 activation.²³ Perhaps the most likely signaling pathway regulating hsp70 expression by cyclic strain stress is Ras→PI3K→Rac→PAK→?→HSF1→hsp70 transcription (see Figure). Based on the results of Xu et al,¹⁵ it cannot be ruled out that the JNK/SAPK pathway mediates the effects of

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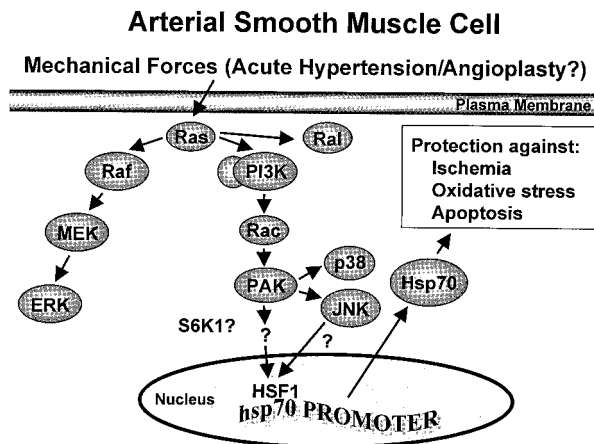
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Hypothetical model of the intracellular signaling pathway responsible for hsp70 accumulation in arterial SMCs subjected to cyclic strain stress. The signaling pathway is shown to involve the small G protein Rac and, to a lesser extent, Ras. A role for both Ras and Rac can be explained by a hypothetical model in which cyclic strain stress activates Ras. Ras then activates PI3K and Rac. Rac in turn induces activation of PAK and JNK/SAPK that may lead to activation of HSF1. Rac also activates other downstream signals that may be responsible for activation of HSF1 and subsequent hsp70 gene expression, including other PAK targets or possibly the p70 S6 kinase (S6K1), which can be activated by Rac.³⁰ Induced hsp70 protects cells against various deleterious stimuli, such as ischemia and oxidative stress.

PAK on HSF1, although there is indirect evidence to suggest that JNK phosphorylation of HSF1 may repress its activity.⁷ On the other hand, oxidative stress-induced hsp70 expression is inhibited by a dominant-inhibitory JNK1 mutant,²⁴ supporting a role for JNK in the pathway leading to hsp70 expression. PAKs also activate downstream signaling targets unrelated to JNK/SAPK.^{25,26} Interestingly, PAK was recently shown to protect cells from apoptosis,²⁷ which suggests that this pathway could mediate cell survival. Another possibility is that the small G protein Ral, which is activated by Ras,¹⁹ affects HSF1 and hsp70 expression through an unknown pathway.

A word of caution is warranted concerning studies in which the major conclusions are based on cells overexpressing dominant-negative mutants of small G proteins. Interpretation of the results is hampered by the fact that small G proteins often share GEFs, and dominant-negative mutants can interfere with activation of other G protein family members through binding to GEFs. Thus, it is possible that in the study by Xu et al,¹⁵ overexpression of N17 Ras and N17 Rac resulted in inhibition of other small G proteins in addition to Ras and Rac. For example, R-Ras, which preferentially activates the PI3K pathway,²⁸ is likely to be inhibited by N17 Ras because this mutant forms a nonproductive complex with exchange factors used by both Ras and R-Ras.²⁹

Future Directions

Induction of hsp70 has been shown to protect various cell types against apoptosis and necrosis, enhancing cell survival after exposure to cytokines, endotoxin, oxidative stress, ischemia, starvation, and other harmful stimuli.¹⁰ Interest-

ingly, hearts from transgenic mice that overexpress human hsp70 are protected against ischemia, have increased recovery of ATP stores compared with littermates without the transgene, and have a reduced infarct size after ischemia and reperfusion.¹ In the study by Xu et al,¹⁵ it is shown that cyclic strain stress increases SMC survival after exposure to oxidative stress, an effect that may be mediated by hsp70. Thus, induction of hsp70 may be beneficial in many different types of cardiovascular disease. However, many questions remain to be answered. For example, what Ras- and Rac-dependent signaling pathway activates HSF1 in SMCs, and does the same signaling pathway induce HSF1 in other cell types and after exposure of cells to other stress stimuli? How does hsp70 protect cells against apoptosis? Is there functional specificity among members of the hsp family? What is the physiological relevance of hsp70 induction in vivo, and does it occur in humans with hypertension and other types of cardiovascular disease? We are likely to see more exciting advances in this area of cardiovascular research in the years to come.

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