REVIEWS

Heat-Shock Proteins and Cardioprotection

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Here we summarized the results of our studies and the data on the role and protective effects of heat-shock proteins, mechanisms of activation of their synthesis, and the role of nitric oxide in this process. The role of heat-shock proteins in preconditioned and adaptive cardioprotection and the possibility of their use as prognostic criteria in cardiology are discussed.

Key Words: *heat-shock proteins; preconditioned and adaptive cardioprotection; nitric oxide; myocardial infarction*

History, Main Terms and Classification

All known organisms (from prokaryotes to higher eukaryotes) display different reactions to environmental stress stimuli. Rapid synthesis of heat-shock proteins (HSP) and a decrease in the production of the majority of other cellular proteins are common characteristic features of the cellular response to stress [35].

The term "heat-shock proteins" arose from the fact that these proteins were initially found in cells subjected to an acute heat procedure. Studies performed at the International Laboratory of Genetics and Biophysics (Naples) in the early 1960s demonstrated that a short-term increase in temperature induces the formation of puffs (localized regions of swelling) in certain chromosomes of salivary glands of *Drosophila melanogaster* [31]. Twelve years later, the expression of genes coding for HSP was shown to be increased in the regions of formation of heat-induced puffs [34].

The term "heat-shock proteins" is not absolutely correct because the synthesis of these proteins was shown to be activated by cold stimuli [13]. Further studies revealed that their synthesis increases under various conditions and treatments [7,11,17,24,32,35]. Therefore, the term "stress proteins" is more correct.

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The following factors stimulate the synthesis of HSPs:

heat;
anoxia, hypoxia, and ischemia;
infections and inflammation;
fever;
UV irradiation;
alcohol;
hyperosmolarity and hyposmolarity;
alkalosis and acidosis;
hydrogen peroxide;
chemotherapeutic agents;
mutagens, carcinogens, and teratogens;
anesthetics;
arsenate and arsenite;
nicotine;
metals (Cd^{2+}, Cu^{2+}, Zn^{2+}, and Pb^{2+});
phenol and its derivatives;
insecticides and pesticides;
denatured and proteins;
and cell cycle, differentiation, and development.

According to their molecular weight, HSPs are divided into 6 main families: high-molecular-weight HSPs (100-110 kDa), HSP90 (83-90 kDa), HSP70 (66-78 kDa), HSP60, HSP40, and low-molecular-weight HSPs (15-30 kDa). Moreover, all HSPs are divided to constitutive HSPs displaying high basal levels and low induction in stress and inducible HSPs. Inducible HSPs are nearly undetectable under normal conditions. However, their synthesis increases sharply in stress.

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HSPs Perform the Function of Molecular Chaperones

Constitutive HPSs are involved in many cellular processes (for example, growth and differentiation, functioning of steroid receptors and tyrosine kinases, and DNA replication). They play a major role in the formation of tertiary and quaternary protein structures [26]. HSP60 and HSP70 bind to those regions of newly synthesized protein chains where undesired hydrophobic interaction and aggregation of protein chains may occur (Fig. 1). HSPs perform the transport of the protein chain to the endoplasmic reticulum, mitochondria, and Golgi apparatus due to free energy derived from hydrolysis of ATP. In these structures, the protein chain is transferred through the membrane to the organelle HSP contributing to the formation of the final subunit structure of the protein.

Thus, HSPs are responsible for regular protein conformations. HSP70 and HSP60 are named chaperones because their functions are similar to those performed by a nursemaid.

HSP70 Provide the Recovery of Damaged Cells

The functions of HSPs in stressed cells have been extensively studied. The total biosynthesis of proteins decreases and HSP70 synthesis increases sharply after stress. The majority of HSP70 are located in the nucleus between stress-induced damaged preribosomes. The content of HSP70 in the nucleus decreases gradually. However, HSP content in the cytoplasm increases. The structure of damaged preribosomes is recovered and total protein biosynthesis reaches the initial level [35].

Thus, HSP70 provide the resistance of cellular mechanisms of the protein biosynthesis to stress. However, the mechanism of this effect is unclear. H. R. B. Pelham hypothesized [28] that stress leads to a partial denaturation of preribosomal proteins (Fig. 2). In this case, hydrophobic regions inducing the aggregation of preribosomes and the formation of insoluble aggregates became opened. HSP70 interact with hydrophobic surfaces, decrease the aggregation of preribosomes,

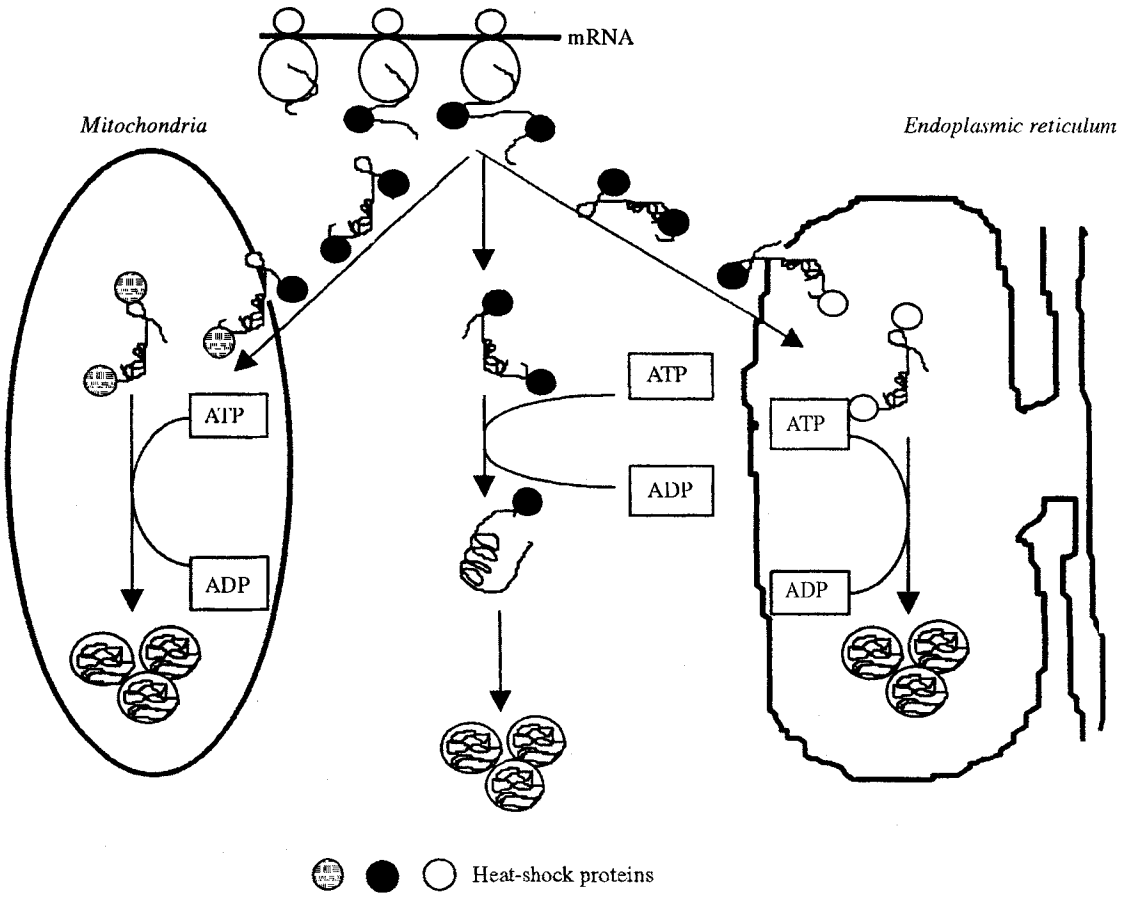

Fig. 1. Involvement of heat-shock proteins in intracellular transport and conformational changes of functionally active structures of newly synthesized proteins.

and attenuate the interaction between damaged proteins. The released protein renatures and resumes its native conformation. This theory postulates that HSP70 are similar to the proteins denatured.

During the poststress phase, HSP70 are accumulated in the cytoplasm [35]. Aggregation and accumulation of denatured proteins, activation of free-radical processes, and calcium overload are the characteristic stress-induced cellular responses. HSP70 can limit these changes because they dissociate anomalous protein aggregates [28], participate in the degradation of irreversibly damaged proteins [27], increase the potency of antioxidant enzymes [30], and decrease the damage-inducing effects of calcium overload by binding to the calcium receptor, calmodulin [33].

The blockade of nitric oxide (NO) overproduction is another mechanism of protective effects of HSP70 [14]. This mechanism is important because the overproduction of NO plays a key role in the development of many diseases (for example, inflammation, hypotension, and ischemic damages) [9,25].

Thus, HSP70 constitute part of the cellular reparation system which protects biosynthetic processes and structural integrity of proteins in the damaged cell.

HSP70 Synthesis is Regulated by Trimerization, Transcription Factor Phosphorylation and Negative Feedback

Regulation of *hsp70* gene transcription is associated with two important elements, a heat-shock consensus element (HSE) and heat-shock transcription factors (HSF). The HSE is a specific element of the *hsp* gene promoter. This element is essential for the activation of *hsp* genes because HSFs bind exactly to the HSE activating RNA polymerase and, therefore, gene transcription.

Under normal conditions HSF are inactive. Stress leads to HSF trimerization (association of 3 molecules). Therefore, HSF become active, enter the nucleus, and bind to the promoter. This stage results in HSF phosphorylation and activation of gene transcription (Fig. 3) [36].

The analysis of regulatory mechanisms of the activation of *hsp70* gene expression showed simultaneous changes of the synthesis of HSP70 and NO. For example, classic inductors of the NO synthesis, lipopolysaccharides [8], activate the synthesis of HSP70 [37]. On the other hand, heat shock, a traditionally used method for the activation of the HSP70 synthesis, is accompanied by an increase in the blood NO content [16]. These data indicate that NO can be involved in the activation of the HSP70 synthesis. Our studies performed in collaboration with Dr. E. B. Manukhina and Prof. A. F. Vanin tested this hypothesis. An inhibitor of NO synthetase (Nω-nitro-L-arginine) induced a twofold decrease in the accumulation of HSP70

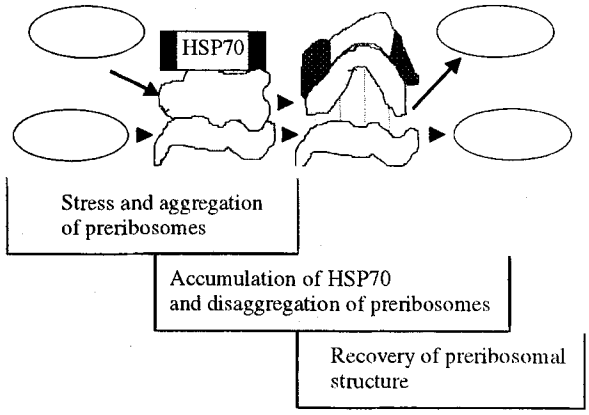

Fig. 2. Role of heat-shock proteins in the inhibition of aggregation and denaturation of damaged proteins.

in organs of rats subjected to a heat-shock procedure (Fig. 4) [20]. An NO donor (iron dinitrosyl complex) induced the accumulation of HSP70 in cell culture (Fig. 5) [19]. These results indicate that NO is involved in the activation of the HSP70 synthesis.

Nitric oxide, a universal physiological regulator [9], is a potent catalyst for the formation of disulfide bonds. Therefore, we assumed that NO catalyzes trimerization by increasing the rate of formation of disulfide between HSF molecules. Chemically, NO^+ interacts with SH-groups of HSF with the formation of S-nitrosothiol. This compound interacts with SH-group of another HSF and forms rapidly a disulfide bond.

Under normal conditions, HSP70 are associated with their own transcription which inhibit trimerization and, therefore, HSF activation (Fig. 3). In stress, HSP70 bind to denatured proteins and release HSF which undergoes trimerization and activates the synthesis of HSPs. This increases the content of HSP70 which then bind to HSF and inactivate it. Therefore, the *hsp70* gene expression terminates. Thus, the interaction between HSP70 and HSFs forms the basis for the mechanism of the HSP70 synthesis autoregulation (negative feedback).

Increasing interest in the medical aspects of this problem is due to the understanding of biological importance of HSPs (for example, their role in the pathogenesis of ischemic heart disease). Two possibilities have been extensively studied: 1) preconditioned and adaptive activation of the HSP70 synthesis can protect the heart from ischemia and reperfusion and 2) HSP70 can serve as diagnostic and prognostic criteria for ischemic heart disease.

HSP70 and Preconditioned Cardioprotection Against Ischemia and Reperfusion

Ischemic heart damage is associated with activation of free-radical processes, calcium overload, protein de-

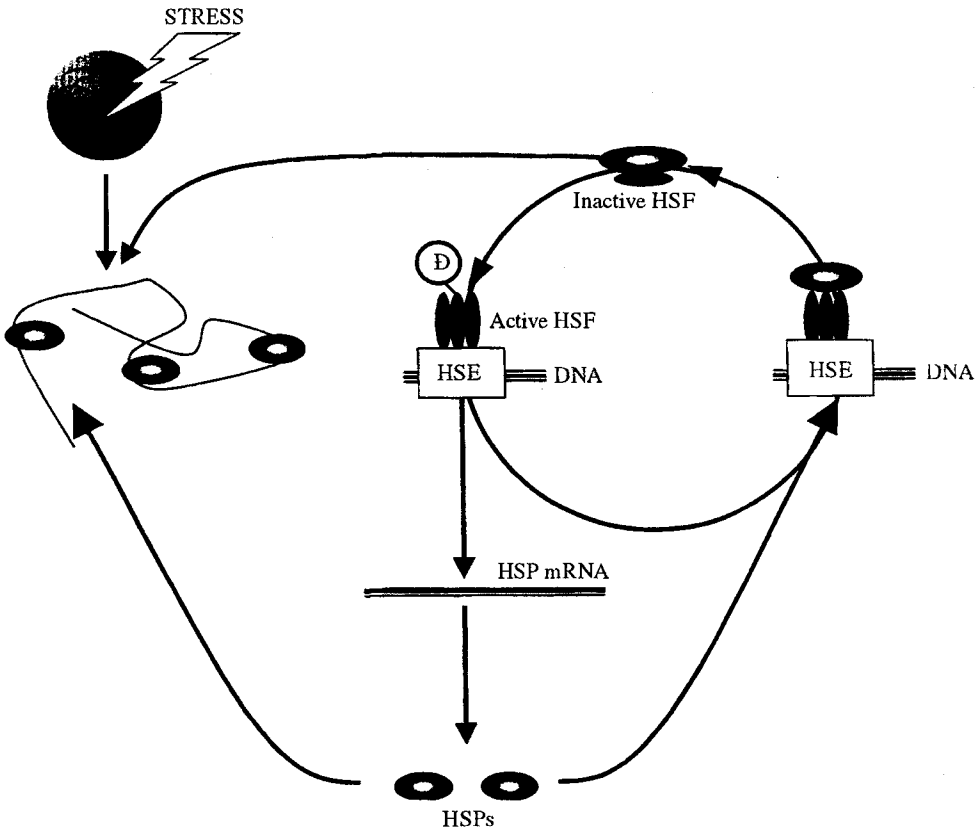

Fig. 3. Mechanism of regulation of the synthesis of heat-shock proteins.

naturation, depletion of ATP and glucose resources, accumulation of toxic metabolites, and cellular pH decrease. All these factors contribute to the activation of the HSP70 synthesis [18]. Studies of ischemic preconditioning showed the significance of the stress-induced HSP70 synthesis. Short-term a few minutes) ischemia inducing the accumulation of the heart increased considerably myocardial resistance to further long-term ischemia [12,26]. A similar effect was observed in heat preconditioning when the synthesis of HSP70 was preliminary stimulated by heat shock [10,11].

These studies indicate that the activation of HSP70 by damage-inducing factors the basis for myocardial adaptation.

There are considerable limitations in the use of ischemic or heat preconditioning in clinical practice.

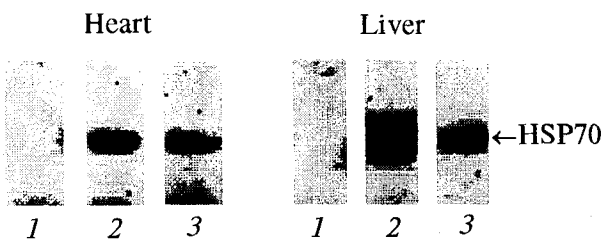

Fig. 4. Inhibitor of NO synthetase (Nw-nitro-L-arginine, L-NNA) inhibits heat shock-induced accumulation of HSP70: *1*) control; *2*) heat shock; and *3*) heat shock+L-NNA.

Therefore, the search for pharmacological and molecular biological methods for the HSP70 activation has attracted much attention. Many medicines (for example, aspirin and nonsteroidal anti-inflammatory drugs) elicit therapeutic effects by decreasing the temperature threshold of the HSP70 synthesis activation [26]. The search for new preparations for the activation of the HSP70 synthesis must be directed to elaborating the drugs which would selectively activate the *hsp70* genes in the heart. However, their action must not be accompanied by cell damage and adverse side effects.

The use of gene therapy seems to be promising for the increase in HSP70 synthesis and heart resistance to ischemia. Genetic engineering can provide directed transfer of the *hsp70* gene with a high expression rate into myocardial cells. Transgenic mice with constantly high rates of HSP70 expression in the myocardium [21,29] displayed an increased heart resistance to ischemia and reperfusion without visible side effects and impaired growth and development.

HSP70 and Adaptive Cardioprotection

Various methods of adaptive medicine which have been elaborated by Prof. F. Z. Meerson and disciples over the last 30 years can provide an alternate approach to cardioprotection. Adaptation of animals to

periodic moderate stress was shown to increase heart resistance to ischemia and reperfusion and toxic concentrations of catecholamines and calcium. Various cellular structures of the heart (mitochondria, elements of the sarcoplasmic reticulum, and nuclei) differ in extremely high resistance to autolysis [1-5,22]. The contents of mRNA and HSP70 in the heart in adapted animals are increased [5].

The content of HSP70 which accumulated in the myocardium during the adaptation to periodic hypoxia was higher than that accumulated during the adaptation to stress. Therefore, several protective effects typical of the adaptation to stress were not observed during the adaptation to hypoxia [23]. Moreover, an inhibitor of the HSP70 synthesis quercetin prevented the development of the heart adaptive resistance to postischemic reperfusion. These data suggest that the activation of the HSP70 synthesis plays an important role in the protective effects of adaptation to stress. This hypothesis was confirmed by the results of our experiments on animals with various resistance to stress.

The increase in myocardial resistance to stress during adaptation was shown to depend on the ability of animals to accumulate HSP70 in response to stress stimuli. Wistar rats accumulated significant amounts of five HSP70 isoforms in the myocardium during the adaptation to periodic stress. Therefore, their heart resistance to damage-inducing factors increased. August rats did not accumulate HSP70 under the effect of periodic stress stimuli, and their heart resistance to damage-inducing factors did not change [2].

Now the mechanisms of HSP70 activation and its role in adaptive cardioprotection can be compared with a "black box". It is clear that HSP70 are involved in three important mechanisms of adaptation and protective effects (Fig. 6). The first mechanism is that HSP70 can act as nuclear signals in the activation of the late genes coding for of structural proteins and enzymes (for example, Ca^{2+}-ATPase and antioxidant enzymes) [15]. The activation of the late structural genes contributes to the resistance of organs. The second mechanism is associated with the involvement of HSP70 genes in the regulation of conformation and intracellular transport of newly synthesized proteins [6,26]. This is important under conditions of a considerable increase in the protein synthesis during adaptation. The mechanism is associated with protective of HSP70.

Generally, the accumulation of HSP70 is an important component of adaptive mechanisms of cardioprotection against damage-inducing effects.

HSP70 as Prognostic Criteria for Myocardial Infarction

It seemed to be apparent that if HSP70 play an important role in cardioprotection against the ischemic damage, the ability of the body to activate the synthesis of these proteins can serve as a prognostic criterion for ischemic heart disease, myocardial infarction, and complications in coronary shunt surgery. In collaboration with Drs. L. Yu. Golubeva, T. A. Zenina (Institute of General Pathology and Pathophysiology), O. Zadorozhnaya, and Prof. V. T. Ivashkin (Moscow Medical Academy), we examined patients with myocardial to test this hypothesis. It was impossible to assess the accumulation of HSP70 in the myocardium in unoperated patients. This made clinical observations difficult. Therefore, we studied peripheral blood lymphocytes. Isolated lymphocytes were placed in a RPMI-1640. Part of the cells was incubated at 37°C. Other cells were incubated at 42°C for 1 h. Basal and heat shock-induced levels of HSP70 were measured. Figure 7 shows the results of Western blot analysis of the contents of HSP70 in lymphocytes of healthy individuals and patients with myocardial infarction which were examined during the first hours after the incidence of infarction. The band width and the staining intensity reflect HSP70 accumulation. Lymphocytes of healthy individuals exhibited moderate basal and heat shock-induced levels of HSP70 (Fig. 7, *a*). Basal and heat shock-induced syntheses of HSP70 were higher in the first group of patients with acute myocardial infarction (Fig. 7, *b*). However, these synthetic processes were not observed in patients of the second group (Fig. 7, *c*). We compared these with clinical characteristics of myocardial infarction. Patients of the second group had more favorable prognosis that those of the first group. All fatal cases (5 out of 15) were occurred in the first group.

It seems unclear why the decrease in basal and stimulated contents of protective HSP70 in patients lymphocytes during the first hours after infarction is associated with a favorable prognosis, where as an increase in these levels is associated with a poor prognosis. Our data not explain this phenomenon.

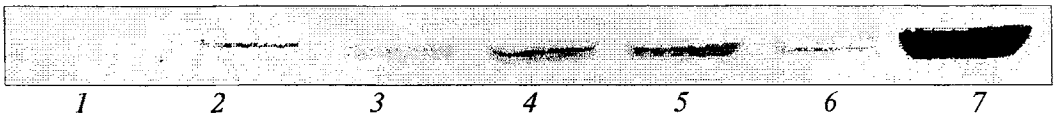

Fig. 5. NO donor (iron dinitrosyl complex, IDNC) induces the HSP70 synthesis in the cell culture: *1*) control; *2-5*) 4,8, 12, and 24 h after the administration of IDNC (100 μM); *6*) 24 h after administration of IDNC (20 μM); and *7*) heat shock.

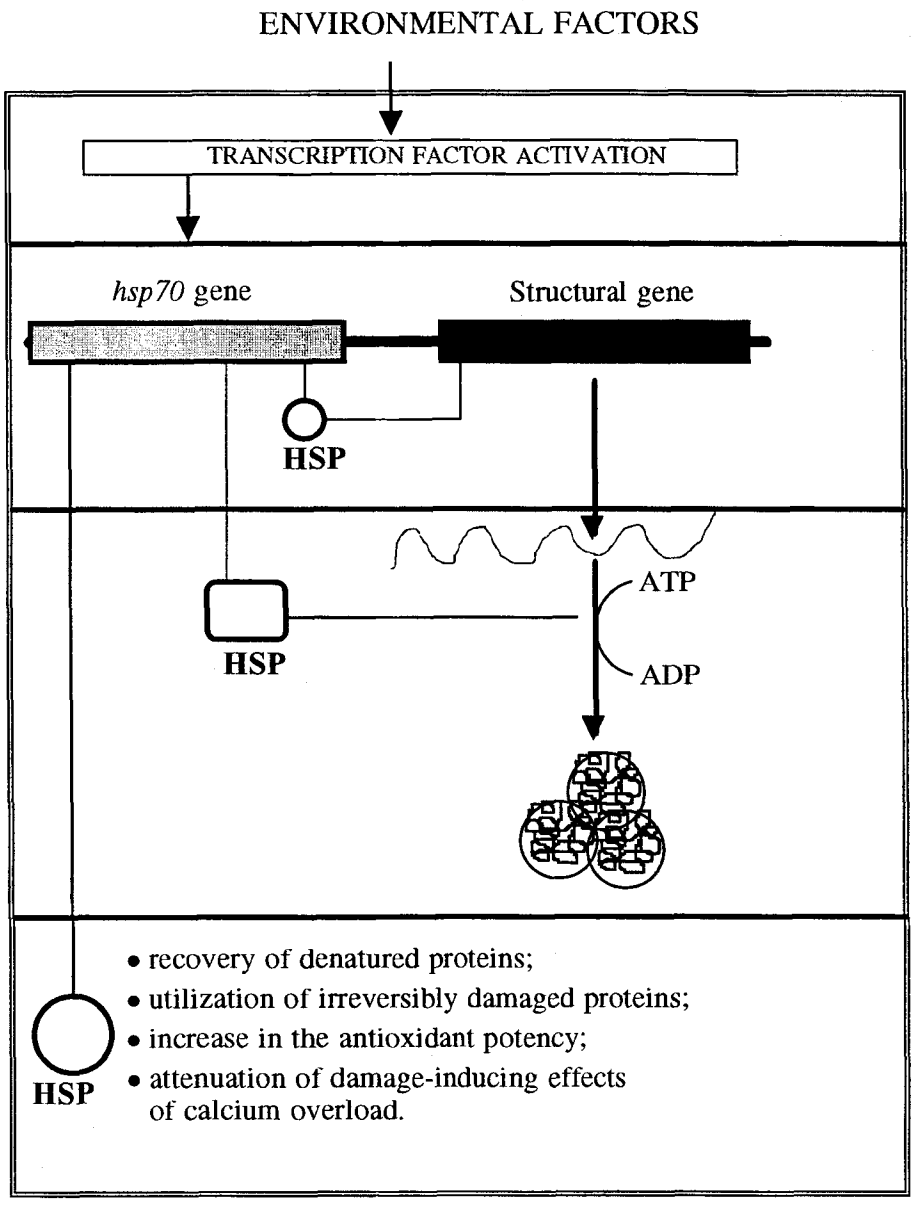

Fig. 6. Role of heat-shock proteins at the main stages of adaptive cardioprotection.

A moderate physiological activation of HSP70 is believed to proceed in lymphocytes of healthy individuals due to the presence of various stress hormones (for example, corticosteroids and catecholamines) in the blood.

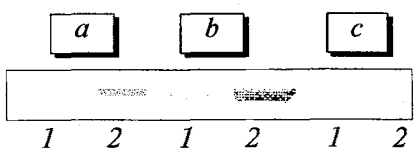

Fig. 7. HSP70 contents in lymphocytes in *a*) healthy individuals and patients with *b*) poor and *c*) favorable prognoses of myocardial infarction. *1*) Basal and *2*) heat shock-induced contents of HSP70.

During the first hours, myocardial infarction is accompanied by pain, a considerable stress reaction, an increase in blood contents of stress hormones, activation of free-radical processes, and denaturation of cellular proteins in organs and blood cells. The data suggest that intense stress reaction is accompanied by severe damage to cardiomyocytes and a poor prognosis of myocardial infarction. On the other hand, the activation of the HSP70 synthesis in lymphocytes is more pronounced under these conditions. Figure 3 shows a possible mechanism of this activation.

It is not inconceivable that in patients with myocardial infarction protective mechanisms that decrease negative effects of stress reaction at the cellular level are activated over the first hours. This limits free-

radical processes, denaturation of intracellular proteins, and, therefore, activation of HSP70 synthesis in lymphocytes. Moreover, this is associated with increased resistance of heart cells and more favorable prognosis of myocardial infarction.

Thus, our studies have shown that lymphocytic HSP70 can serve as markers of intensity of stress reaction that accompanies myocardial infarction during the first hours. Obviously, the use of HSP70 as prognostic criteria for myocardial infarction requires further studies.

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