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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

# Heat Stress Induces Formation of Cytoplasmic Granules Containing HSC70 Protein

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**Abstract**—Using indirect immunofluorescence, in this study we showed that the constitutive heat shock protein HSC70 forms granule-like structures in the cytoplasm of human cells several days after the exposure to heat stress. It was shown that this effect is not the result of HSC70 overexpression under heat stress and is not due to the formation of hyperthermia-induced translational stress granules in the cytoplasm.

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Hyperthermia (heat stress) is a strong cellular stress. Hyperthermia causes denaturation and aggregation of proteins, leading to the reorganization of the cytoskeleton, fragmentation of the endoplasmic reticulum and Golgi apparatus, disturbance of RNA splicing, etc. To prevent fatal consequences, cells start the expression of highly conserved proteins known as heat shock proteins (HSPs), which are involved in maintaining the protein homeostasis [1]. Heat shock proteins of the HSP70 family implement protective functions in all living organisms from archaea to humans. The implementation of protective and many other functions of HSP70 is due to its chaperone activity [2]. One of the functions of chaperones is that they bind to damaged or newly synthesized polypeptides and help them to acquire the native conformation [3]. Chaperones are able to find the hydrophobic regions in the target polypeptide that are open in damaged proteins or can be opened in normal mature cellular proteins as a result of change in their conformation [4]. Such conformational changes occur not only under stress but also as a result of cascade modifications of proteins during intracellular signaling. Thus, proteins of the HSP70 family are one of the key elements of the quality control systems of proteins involved in the life support systems of all cells and are the first line of cell defense in heat stress and other types of stresses. In mammalian cells, several members of the HSP70 family can be detected simultaneously. The main ones are the inducible HSP70 (HSPA1A/B), the constitutively synthesized HSC70 (HSPA8), the glucose-regulated protein GRP78 (HSPA5), and mortalin (HSPA9), which is located in mitochondria [5].

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The majority of proteins of the HSP70 family are located in the cytoplasm [4, 6]. Under stress, some of them can be dynamically reallocated to cellular compartments. For example, the HSP70 protein in hyperthermia is translocated to the nucleus and accumulates in nucleoli, presumably ensuring the stability of ribosome assembly [7]. Nevertheless, these events frequently occur not at the time of stress (for example, in hyperthermia) but several hours later, which indicates a significant difference in short-term and long-term effects for these proteins.

Despite the fact that the role of heat-shock proteins in the cell response to stress has been studied for several decades, the short-term effects that appear immediately or several hours after stress remain to be most comprehensively studied in hyperthermia. Much less is known about the role of heat shock proteins in the long-term effects of hyperthermia, which manifest themselves several days after exposure to stress. The aim of this study was to investigate the nature of long-term effects of hyperthermia.

One of the approaches to studying the functions of a protein is the analysis of its intracellular distribution. Intracellular localization is an important factor determining the function of a protein and its interaction partners. It was of interest to analyze the localization of HSP70/HSC70 proteins in human cells cultured for several days after heat stress. For this purpose, MCF-7 cells (human breast adenocarcinoma) in the exponential growth phase were incubated at 45.5°C for 30 min. After the incubation, the cells were grown for 3 days under normal physiological conditions and then fixed with methanol and immunostained with mouse monoclonal antibodies against HSP70/HSC70 (Abcam, Great Britain). The results of these experiments are shown in Fig. 1a. It can be seen that, in the control cells, HSP70/HSC70 proteins are uniformly distributed over the cytoplasm and that heat stress at 45.5°C for 30 min caused no significant changes in the protein

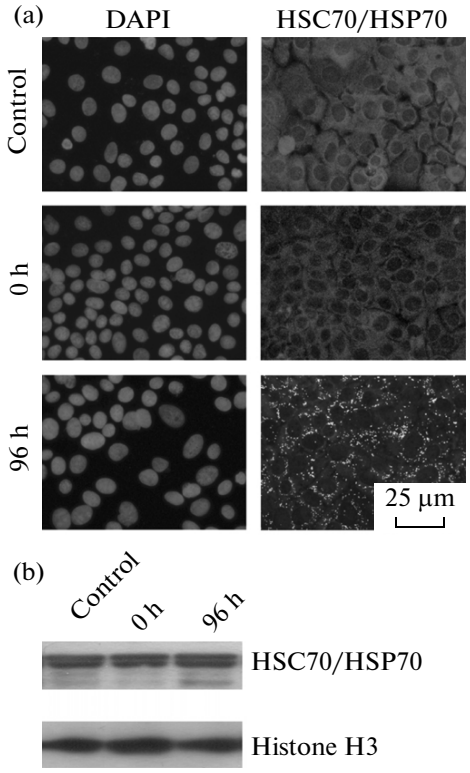


**Fig. 1.** Heat stress induces the formation of cytoplasmic granules containing heat shock proteins. (a) Immediately after the heat shock (45°C, 30 min) and at the specified time intervals after the heat shock cells were fixed and stained with the antibodies against HSP70/HSC70. DNA was stained with DAPI. Here and in Figs. 2 and 3, micrographs were taken using a Zeiss Axio Scope A1 fluorescence microscope (Karl Zeiss, Germany). (b) Western blot analysis of HSP70/HSC70 in MCF-7 cells (control cells and cells exposed to heat shock after the specified time intervals). Histone H3 was used as an internal control.

localization. However, three days after the exposure to stress, the pattern of cytoplasmic distribution of HSP70/HSC70 in many cells changed dramatically. By this time, HSP70/HSC70 proteins aggregated to form sufficiently large granule-like cytoplasmic structures. This effect was characteristic of not only MCF-7 cells. Similar results were obtained with HeLa cells (data not shown).

We assumed that such redistribution may be caused by the stress-induced overexpression of HSP70/HSC70 proteins. To test this assumption, we compared the total content of HSP70/HSC70 proteins in the control cells and in the cells subjected to heat shock (immediately after stress and 3 days later) using Western blot analysis. The results of these experiments indicate that the expression of HSP70/HSC70 is induced by heat stress; however, 3 days later the expression returned to the control level (Fig. 1b). Thus, changes in the intracellular content of HSP70/HSC70 proteins during and after heat stress cannot be the cause of the observed effect of formation of cytoplasmic HSP70/HSC70-containing granules.

In previous experiments, immunostaining and Western blot analysis were performed using the antibodies that recognized two proteins—inducible HSP70 and constitutive HSC70. Despite the fact that they are homologous by 85%, their functions in some cases significantly differ [8]. With this in mind, we decided to determine whether the cytoplasmic granules contained both of these proteins or only one of them. For this purpose, we used the antibodies that recognize only the HSP70 protein. First, we confirmed that the antibodies that recognize both HSP70/HSC70 proteins showed the presence of two protein bands on Western blots (Fig. 2a). The upper and lower bands corresponded to the HSC70 and HSP70 proteins, respectively. The antibodies recognizing only the HSP70 protein stained only one band.

Having made sure that HSC70 and HSP70 proteins can be distinguished using the antibodies, we performed a double immunocytochemical staining of MCF-7 cells 3 days after the exposure to heat stress using the antibodies recognizing one protein (HSP70) and the antibodies recognizing both proteins (HSP70/HSC70). The results presented in Fig. 2b show that the cytoplasmic granules formed on the third day after stress were recognized only by the antibodies against both proteins HSP70/HSC70. The nature of the distribution of the HSP70 protein remained diffuse. Thus, it is clear that primarily the constitutive protein HSC70 is involved in the formation of cytoplasmic granules under heat stress.

It is known that the exposure to heat stress may lead to the formation of stress granules in the cytoplasm of cells, which ensure translational inhibition and polysomes disassembly [9]. Such structures include the canonical translation initiation factors, particularly the eIF4G factor, which binds to the small heat shock protein HSP27 in the granules, thereby ensuring the inactivation of the cap-binding complex [10].

However, there is no evidence that the translational stress granules contain the proteins of the HSP70 family. At the same time, translational stress granules contain the small ribosomal subunit, translation initiation factors (phosphorylated eIF2α, eIF3, eIF4G, eIF4E, and PABP1), as well as other RNA-binding proteins [9]. The latter include the ARE-binding proteins HUR, YB-1, and TTP and two related RNA-binding proteins TIA-1 (T-cell intracellular antigen) and TIAR (TIA-1-related). Despite the fact that the "classic" translational granules undergo disassembly within several hours after stress, we decided to test whether the HSC70-containing cytoplasmic structures discovered by us can be translational stress granules with a delayed (for any reason) dissociation time.

To test this assumption, the control MCF-7 cells, the cells subjected to heat shock, and the cells subjected to heat shock and then cultured for 3 days under normal conditions were immunostained with the antibodies against the YB-1 protein. The results of this series of experiments are shown in Fig. 3. It can be

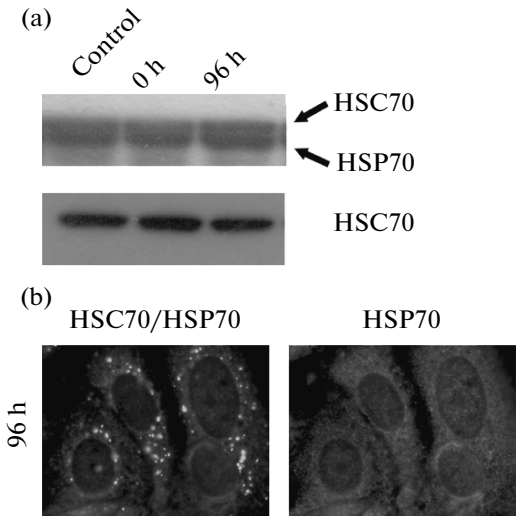


**Fig. 2.** Only the HSC70 protein is involved in formation of cytoplasmic granules after heat stress. (a) Western blot analysis using the antibodies against HSP70/HSC70 and HSP70 in the control MCF-7 cells and the MCF-7 cells subjected to heat shock after the specified time intervals. (b) The distribution of HSP70/HSC70 proteins and HSP70 protein in MCF-7 cells 3 days after the heat shock (fixation and simultaneous staining with the antibodies against both HSP70/HSC70 and HSP70).

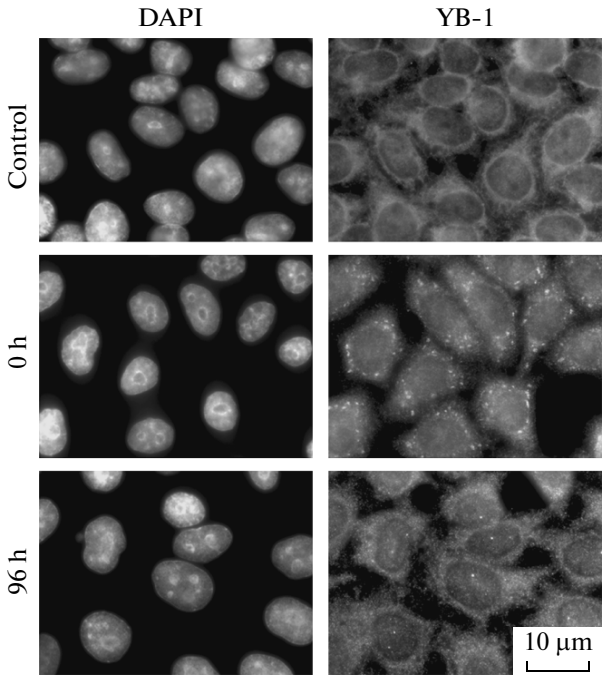


**Fig. 3.** Translational granules containing the YB-1 protein are formed immediately after heat shock but are absence in cells 3 days after stress. The control MCF-7 cells and the MCF-7 cells subjected to heat shock after the specified time intervals were fixed and stained with the antibodies against YB-1. DNA was stained with DAPI.

seen that the translational stress granules marked with YB-1 are formed in the cytoplasm of cells immediately after heat shock but not in the cells 3 days after the exposure to stress. This result was also confirmed using the antibodies against other protein markers of translational stress granules, in particular eIF4G and PABP1 (data not shown). On the basis of this fact, it can be concluded that the HSC70 protein-containing cytoplasmic structures observed by us are not the "classical" stress granules involved in the translation termination.

Thus, we have shown that, several days after acute heat shock, granule-like structures containing the HSC70 proteins appear in the cytoplasm of cells. The nature of these structures is still obscure; however, it is obvious that they are not related to the translational stress granules. It is known that heat shock proteins can aggregate in certain subcellular compartments at the time of stress and thus ensure their architectural integrity and functional activity. In this regard, it can be assumed that the HSC70-containing structures discovered by us may be associated with certain subcellular compartments damaged during the heat stress. Testing this hypothesis will be the objective of our future research.

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