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Major stress protein Hsp70 interacts with NF-kB regulatory complex in human T-lymphoma cells

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Abstract Polypeptides belonging to the Hsp70 major stress protein family and to the NF-kB/Rel multi-functional regulatory complex are known to be involved in cellular defense mechanisms. It was suggested that both systems may interact in cells that respond to injuring stimuli. To check this, Molt4 human lymphoma cells were heated at 43°C for 15 min and, after a 6 h post-shock recovery period, the cells were activated with phorbol ester or bacterial lipopolysaccharide. It was found that mild heat shock caused a substantial increase of the intracellular Hsp70 content with the concomitant suppression of NF-kB complexes, though the latter was properly activated in non-stressed cells. After a 24 h period of being inactive the complex fully recovered its activity and p65 and c-Rel subunits migrated to the nucleus. This new active period lasted even longer than that in non-heated control cells. As this suggested the existence of a Hsp70-related mechanism of NF-kB/Rel complex retention in cytoplasm, we carried out immunoprecipitation with the use of anti-Hsp70 and anti-Rel antibodies. All three Rel family members p65, c-Rel, p50, but not their precursors and IkBα inhibitory protein were shown to co-precipitate with the stress protein and anti-Hsp70 antibodies from both heated and non-heated cells. We conclude that the Hsp70 stress protein may confer a new mechanism of NF-kB regulation in cells affected by elevated temperature or other factors related to the cellular response to stress.

INTRODUCTION

Two protein systems among others are activated in the course of cell responses to pathogenic stimuli or toxins, heat shock proteins and NF-kB/Rel regulatory complex. The NF-kB system consists of protooncogenes belonging to the Rel protein family: p50, with its precursor p105, p52, its precursor p100, c-Rel, p65 (RelA) and B-Rel; these polypeptides are associated with IkB inhibitory proteins (Grilli et al 1993; Thanos and Maniatis 1995). It seems important that the activation of NF-kB complex does not need any additional protein synthesis; all the components occur in the cytoplasm. Stimulation of the complex with a certain factor causes the release of Rel family members from IkB inhibitory polypeptides and transportation of homo- or heterodimers consisting of NF-kB subunits to the cell nucleus; their final targets are specific promoter regions of DNA (Baeuerle and Henkel 1994). Both the NF-kB complex and IkB proteins undergo proteolytic modification (Baldwin 1996). The regulation of the whole complex takes several minutes from the stimulation of surface receptor or intracellular effector until the target DNA sequence is bound by Rel family proteins.

DNA sites that are controlled by NF-kB represent a great variety of genes including interleukin-2, 6, granulocyte/macrophage-colony stimulating factor, MHC class I, NO synthase and many others. A long list of NF-kB stimuli contains phorbol esters, growth factors, virus infection, T-cell activators, TNF, LPS, interleukin-1, the latter referring to well-known mediators of the inflammatory reponse (Baeuerle and Henkel 1994).

Major heat shock protein 70 kDa expression usually related to the stress response in a variety of cells and organisms is also known to be controlled by factors stimulating cell differentiation, growth and death (Feige and Polla 1994; Morimoto et al 1994). These processes transiently

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need new protein synthesis, modification, intracellular transport of polypeptides, assembly of protein structures and, finally, removal of needless or damaged proteins by degradation mechanisms. The members of the Hsp70 protein family, Hsp70 inducible and Hsc70 constitutive components, are implicated in all the above functions; this reflects their particular chaperoning activity (Morimoto et al 1994). It was shown that besides nascent or damaged polypeptides Hsp70 and Hsc70 are able to form complexes with mature cellular proteins. Among those are p53, c-myc, v-Rel and other cellular oncogenes (Brown et al 1994; Henriksson et al 1992; Lim et al 1990) as well as such key cell molecules as actin and calmodulin (Margulis and Welsh 1991; Stevenson and Calderwood 1990).

It is well established that many known inducers and mediators of NF-kB complex including interleukin-1β, LPS, Newcastle Disease Virus and others are able to up-regulate expression of the Hsp70 gene (Welsh et al 1991; Zhang et al 1994; Collins and Hightower 1982; Cheung and Dosh 1993). Hsp70 has been implicated in protection against lipid peroxidation and against the toxic effect of nitric oxide in beta-cells, and both phenomena are indicative of responses to pathogenic stimuli (reviewed in Jackier-Sarlin et al 1994; Bellmann et al 1996).

It is noteworthy that heat shock, together with the induction of stress proteins, can suppress activity of NF-kB (Kretzremy and Arrigo 1994). Feinstein and co-authors demonstrated that heat shock or transfection of glial cells with the Hsp70 gene caused suppression of inducible NO synthase; this effect was directly associated with the inhibition of NF-kB activity (Feinstein et al 1996). This evidence and the demonstration of the binding of v-Rel protein to Hsc70 (Lim et al 1990) led us to assume that the NF-kB complex subunits and Hsp70 family members may interact physically in cells activated by an appropriate factor. In order to check this possibility we produced, by mild heat shock, large quantities of Hsp70 in Molt4 human lymphoma cells. We found that alongside the process of Hsp70 accumulation, NF-kB complex, though stimulated with phorbol ester or LPS, persisted in inactive form for hours; becoming active again thereafter. Finally, the binding of p65 and other Rel family members to Hsp70 was demonstrated, suggesting the possible role of Hsp70 in the temporary down-regulation of the complex during the acute phase of the inflammatory process.

MATERIALS AND METHODS

Materials

Cell culture reagents (DMEM/F12, gentamicin) were from Gibco BRL (Gaithersburg, MD, USA), fetal calf serum, from Fundação Bio-Rio (Rio de Janeiro, Brazil). Bacterial lipopolysaccharide (LPS) *Escherichia coli*, serotype 026: B6, phorbol-12-myristate 13-acetate (PMA) and nitrocellulose membrane were purchased from Sigma (St Louis, MO, USA); CNBr-Sepharose 4B and Protein A-Sepharose were from Pharmacia (Uppsala, Sweden). The 3B5 monoclonal antibody to Hsp70 obtained in our lab recognized only inducible Hsp70 isoform in Western blotting and immunoprecipitation assays. A panel of antibodies recognizing RelA (p65), c-Rel, p50, p105 precursor and IkBα inhibitor proteins all belonging to NF-kB/Rel complex (Rice et al 1992) was provided by Dr N.Rice, NCI Frederick Cancer Research & Development Center, Frederick, USA.

Cell treatment

Human T-lymphoma Molt4 cells were grown in DMEM/F12 medium supplemented with 10% fetal calf serum and 50μg/ml gentamicin at a presence of 5% CO_2. Heat shock was performed at 43°C for 15 min and the cells were left to incubate at 37°C. At regular intervals after heating, cell samples were taken to study the kinetics of Hsp70 accumulation. In experiments dealing with the combined effect of heat stress and NF-kB stimulators, the cells were first heated at 43°C and then incubated for 6 h at 37°C. Both control and heat shocked cells were induced by 0.16μM phorbol myristoylacetate (PMA) or 10 ng/ml bacterial lypopolysaccharide (LPS). After 1 and 24 h incubation the cells were washed and the nuclei were isolated as described elsewhere (Dignam et al 1983). Briefly, the cell pellet was resuspended in 10mM HEPES, pH 7.9; 10mMKCl; 0.1mM EDTA; 0.1 mM EGTA; 1mM DTT and 0.5mM PMSF. Cells were allowed to swell on ice for 15 min and, after Nonidet P-40 was added to the final concentration 0.6%, they were vortexed and spun. After washing in the above solution the pellet was dissolved in cold 20mM HEPES, pH7.9; 400 mM NaCl; 1 mM EDTA; 1mM EGTA; 1mM DTT and 1mM PMSF.

Immunochemical procedures

To estimate the kinetics of Hsp70 variation after heat shock, the cell probes were assayed by Western blotting. Transition of p65 protein to the nucleus has also been proved by the immunoblotting analysis of purified cell nuclei. Protein concentration in samples was measured by the dye-binding method (Bradford 1976). The loading volumes were corrected based on the results of a provisional electrophoretic run. Equal amounts of total cell protein were loaded onto gel; after electrophoresis (Laemmli 1970) proteins were transferred to nitrocellulose. Blots were probed with the antibodies to Hsp70, RelA (p65) or c-Rel followed by secondary antibodies conjugated with horseradish peroxidase. Peroxidase activity was finally developed with the aid of a chemiluminescent cocktail.

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To study Hsp70–NF-kB interaction, the following experiments were carried out. The cells were lysed in low RIPA buffer (150 mM NaCl; 2 mM EDTA; 0.5% Triton X-100; 20 mM tris-HCl, pH7.5; 1 mM Na orthovanadate; 50 mM NaF; 1 mM PMSF), and, after the removal of non-specific material with the aid of pre-immune mouse IgG linked to CNBr-Sepharose, lysates were subjected to immunoprecipitation. The latter was performed with the aid of anti-Hsp70 monoclonal antibodies, 3B5 clone, coupled to CNBr-Sepharose or polyclonal antibodies against the NF-kB complex subunits incubated with Protein A-Sepharose prior to the reaction with cell lysate. Non-specific (pre-immune) mouse immunoglobulins linked to CNBr-Sepharose or rabbit IgG bound to Protein A-Sepharose were employed for controls. Immunoprecipitates were subjected to electrophoresis and immunoblotting. The blots were initially incubated with the antibodies against the protein suspected to be in the co-immunoprecipitate, followed by peroxidase-conjugated secondary antibodies and the blots were then developed using a chemiluminescent cocktail. To verify the effectiveness of immunoprecipitation, the blots were also incubated with the antibody employed previously for immunoprecipitation followed by staining with the use of the diaminobenzidine as the chromogene.

Immunofluorescense

For intracellular localization of the p65 subunit of NF-kB complex, both control cells and those which had been heat shocked combined with the phorbol treatment, were plated onto poly-l-lysine, fixed in 4% formaldehyde for 15 min, and permeabilized in 0.1% Triton X-100 for 60 min. Finally, the preparations were incubated overnight at 4°C with the anti-p65 antibody followed by a secondary antibody labeled with FITC. The immunofluorescence pattern was observed with the aid of a Zeiss Axioscop fluorescence microscope. Control incubation with secondary antibodies revealed negligible staining.

RESULTS

Dynamics of Hsp70 accumulation

It has been established that heat stress and other factors triggering cellular defense mechanisms are able to induce stress protein synthesis that quickly peaks and then gradually declines (Mizzen and Welch 1988), while the amount of intracellular heat shock protein 70 kDa flattens out a few hours later and remains at the same elevated level for much longer (Margulis and Welsh 1991). In Molt4 cells optimal conditions for Hsp70 accumulation were found in Western blotting analysis of cell samples taken at given time points after heat shock at 43°C for 15 min. Gradual elevation of Hsp70 content was observed within 12 h following heat shock; after that the protein content gradually lowered (Fig. 1A,B). High protein levels were already found 6 h after heat stress, at which time NF-kB activators were applied. Additionally, heat treatment causing substantial accumulation of Hsp70 had no apparent effect on cell viability and its influence on growth dynamics was also weak (Fig. 1C).

Heat shock delays activation of NF-kB

Two inducers of NF-kB/Rel complex were employed, PMA and LPS, both known to stimulate Rel activity in a

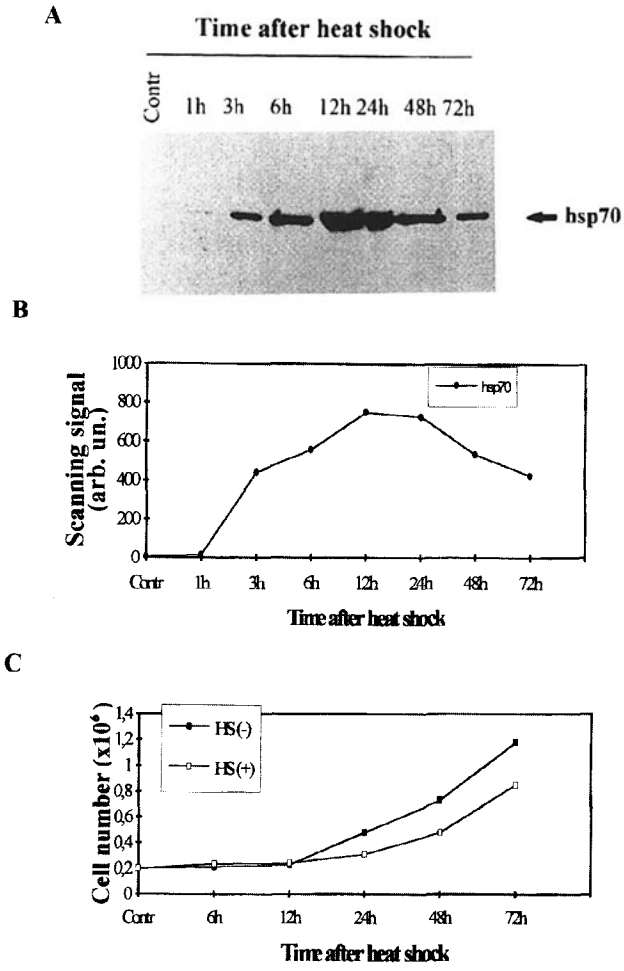

Fig. 1 Kinetics of Hsp70 accumulation in Molt4 cells after heat shock. (A) Following heat shock at 43°C for 15 min cells were recovered at 37°C for indicated time intervals and then lysed; equivalent amounts of protein (50 µg) were subjected to SDS/PAGE and Western blotting with monoclonal 3B5 anti-Hsp 70 antibody. (B) Densitometry profile of blot in A. (C) Cell growth dynamics in Molt4 cells without and after heat shock. The data shown are representative of two separate experiments.

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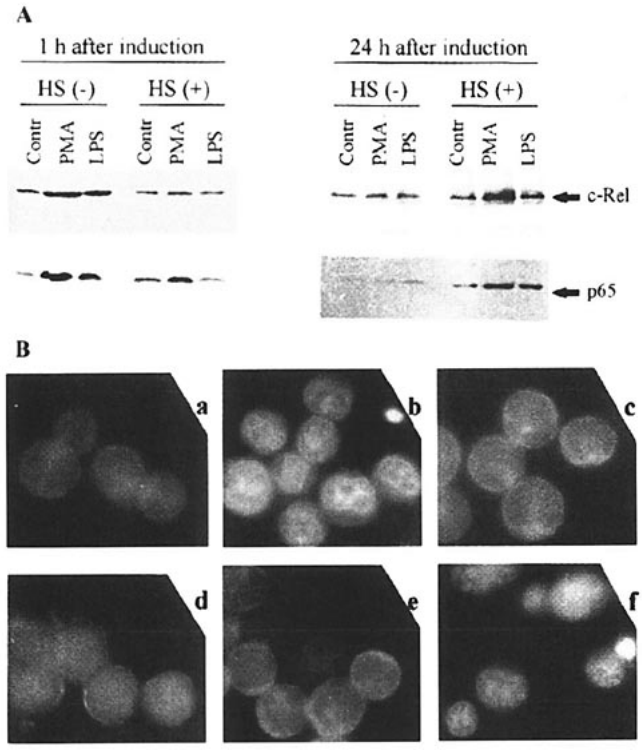

Fig. 2 Heat shock temporarily impedes PMA- or LPS-mediated activation of NF-kB complex; activity of complex restored 24 h later. (A) Western blot analysis of p65 and c-Rel proteins expression in Molt4 cells after combined effect of heat shock and the NF-kB complex inducers. Following heat shock at 43°C for 15 min cells were allowed to recover for 6 h and then incubated with PMA and LPS for 1 or 24 h. Nuclear fractions were purified and equal amounts of protein (100 μg) were subjected to SDS/PAGE and immunoblotting. Blots were probed with the polyclonal antibody to p65 and c-Rel. (B) The cells prepared as in (A) were immunostained with the anti-p65 antibodies. Non-heated (a-c) and preheated (d-f) cells were treated with PMA for 1 h (b, e) or for 24 h (c, f)

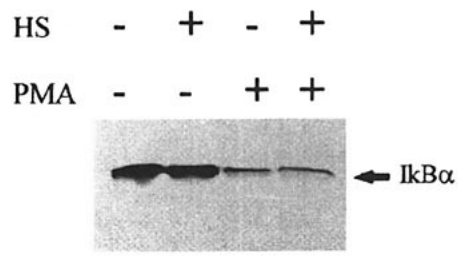

Fig. 3 Western blot analysis of IkBα protein in Molt4 cells stimulated with PMA after heat shock. Molt4 cells were heat-shocked (43°C for 15 min) and allowed to recover for 6 h followed by exposure to PMA for 1 h. Equal amount of protein (100μg/well) from total cell lysates were subjected to SDS/PAGE and immunobloting with the aid of polyclonal antibodies to IkBα.

variety of lymphoid cell lines. A typical experiment included the following steps: mild heat shock, 6 h incubation at 37°C, and finally administration of PMA or LPS. Results of blotting of nuclear fractions with the aid of antibodies to p65 and c-Rel components of NF-kB complex showed that in normal cells response to PMA or LPS developed within 1 h (Fig. 2A). Heat shock strongly affected the time-course of the complex activation: only a small amount of p65 as well as of c-Rel were found in heated cells after induction, as compared with non-heated ones (Fig. 2A). 24 h later in cells incubated with PMA or LPS only traces of NF-kB components occurred in the nuclei, while in cells heated prior to the administration of above inducers p65 and c-Rel were localized to the nucleus. This pattern was demonstrated in immunofluorescence studies with the aid of an anti-p65 antibody. Only background cytoplasmic staining was found in control non-heated and non-induced cells (Fig. 2B). Treatment of non-heated cells with PMA caused almost instant transition of p65 to the nucleus; during the next 24 h the nuclei were cleared of the protein. In heated cells the traces of p65 were found in the cell nucleus 1 h after activation with PMA. The transition of the protein occurred much later and 24 h after NF-kB stimulation one could observe strong staining of the nucleus (Fig. 2B). It is worth mentioning that this new, active state of NF-kB/Rel complex provided by heat shock persisted for the next 24 h, as shown in the immunoblotting results below.

The state of IkBα inhibitory polypeptide in control and induced cells was studied with the aid of Western blotting. The results showed that in control and heated cells IkBα exists in intact form as a polypeptide with a molecular mass of 36 kDa. (Fig. 3). Activation with phorbol ester caused rapid reduction of that band in samples of heated and non-heated cells, though the same amount of total cell protein was loaded on the gel.

Binding of Rel proteins to hsp70

The results suggest that a delay in NF-kB complex activation in heat shocked cells was caused by physical interaction of its components with the Hsp70. This possibility was tested by means of immunoprecipitation combined with the immunoblotting as described in Materials and Methods. In the first series of experiments the assay was performed with the antibody recognizing IkBα inhibitory protein, p65, p50 Rel family member and its precursor p105. According to Western blotting all the immunoprecipitates did not contain Hsp70 (data not shown). When the assay was performed with anti-Hsp70 antibody, p65 protein was found to co-immunoprecipitate with the chaperone, (see Fig. 4). Neither p65 nor c-Rel or p50 was

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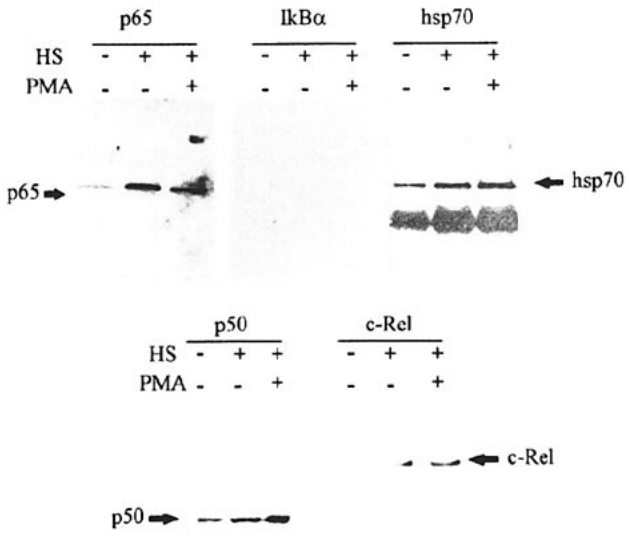
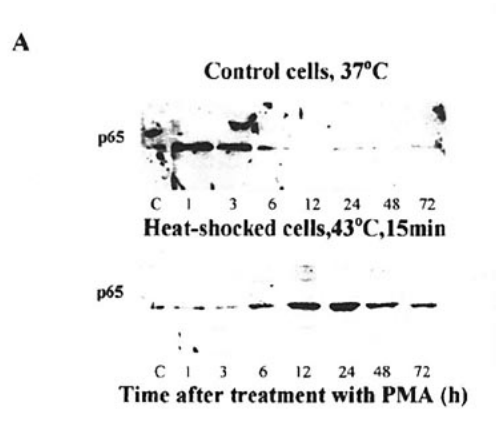


Fig. 4 Interaction between Hsp70 and members of the Rel family. Molt4 cells were heat-shocked (43°C for 15 min) and allowed to recover for 6 h followed by exposure to PMA for 60 min. Total cell lysates (1.5 mg of protein per sample) after depletion with the pre-immune mouse IgG were subjected to immunoprecipitation with anti-Hsp70 antibodies coupled to CNBr-Sepharose. The immunoprecipitates were subjected to SDS/PAGE and Western blotting with the aid of polyclonal antibodies to p65, c-Rel , p50 and IkBα and thereafter with monoclonal anti-Hsp70. No cross-reaction was found between two secondary antibodies.

detected in control cells with non-specific mouse IgG coupled to CNBr-Sepharose. Binding of p65 Rel family members to Hsp70 did not depend on whether the cells were activated by PMA or not. No binding of NF-kB precursors and IkBα protein to Hsp70 was found in any cell samples. The validity of the immunoprecipitation reaction has been proved by testing the membrane with anti-Hsp70 antibody; only the Hsp70 band and that of IgG heavy chain occurred on the blot (Fig. 4).

In order to test the possibility that two other members of the Rel family, p50 and c-Rel, may interact with Hsp70, we also performed immunoprecipitation with the anti-Hsp70 antibody coupled to Sepharose. Samples of normal and heated cells and cells treated with phorbol ester following heat shock were employed for the assay. It was demonstrated that in all cell samples Hsp70 binds p50 and c-Rel proteins (Fig. 4).

In order to analyze in more detail the kinetics of intranuclear transition of p65, the latter was monitored by Western blotting. The data demonstrate that p65 protein appears in the nucleus of non-heated cells within the first hour after stimulation with PMA and then gradually emerges (Fig. 5A). The intranuclear transition of p65 in heated cells was substantially postponed and its content in the nucleus peaked only 24 h after stimulation. The cells persisted in this activated state, characterized by a high content of p65 in the nucleus for more than 24 h (Fig. 5A). To estimate and compare the kinetics of Hsp70 and nuclear p65 content variations, the blots were developed with the aid of a chemiluminescent cocktail and quantified by densitometry. Three curves, one representing part of a plot from Fig. 1B (6–72 h after heat shock) and two others showing the kinetics of nuclear p65 level in control and heat-shocked cells were superimposed on the same plot (Fig. 5B). It appears convincing that 24 h after the cell stimulation with PMA the amount of heat shock protein decreased, while p65 accumulated in nucleus.



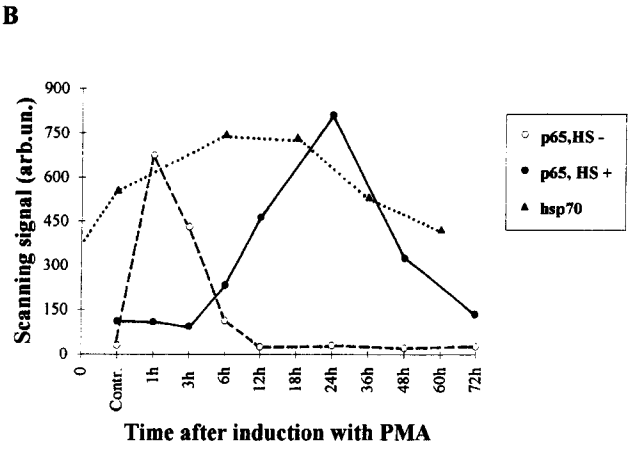


Fig. 5 Nuclear transport of p65 in stressed and control Molt4 cells after induction with PMA. (A) Control cells and heat-shocked (43°C for 15 min) followed by 6 h recovery were incubated with PMA for the indicated time intervals. Nuclear fractions were purified and equal amounts of protein (75 μg) were loaded onto SDS-gel and transferred proteins were probed with anti-p65 antibody. (B) Kinetics of p65 nuclear transition as revealed by densitometric scanning of blots above. The data shown are representative of two separate experiments.

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DISCUSSION

This study was based on the assumption that the major heat shock protein Hsp70 and the components of NF-kB/Rel complex have to interact in cells responding to a certain stressor or to an inflammatory cytokine. The model system chosen for our experiments, comprised of human Molt4 lymphoma cells, was clearly shown to respond to the stimuli for both protein systems. Mild heat shock induced gradual accumulation of major stress protein Hsp70 without a significant effect on cell growth and survival (Fig. 1). The amount of Hsp70 in heated cells was kept high for at least 18 h (for 6–24 h after heat shock). In cells affected by a certain stress factor, Hsp70 is thought to form a dynamic complex with a fraction of cellular proteins including those damaged by a stress, nascent and completed polypeptides, like actin (Ang et al 1991; Beckmann et al 1990; Margulis and Welsh 1991). The structure formed by Hsp70 can persist for a long time, and in pancreatic islets which have been heat-shocked or treated by interleukin-1β, Hsp70 remains inaccessible to specific antibodies and presumably to proteases (Margulis and Welsh 1991). 24 h after heat shock the amount of the protein in Molt4 cells observed on blots began to decline, probably due to a low rate of its synthesis. Two other reasons for loss of Hsp70 may be post-shock recovery of protease activity able to digest Hsp70, and dilution of protein due to cell multiplication.

The adequacy of our model was also demonstrated in experiments dealing with the activation of NF-kB complex. Both activators, LPS and phorbol ester, were found to induce efficiently the translocation of p65 and c-Rel components to the nucleus. The full cycle of NF-kB complex activation including processing, nuclear transition of p65, and finally, depletion, took less than 6 h. In heated cells that process was found to be quite different. Mild heat shock followed by a rapid accumulation of Hsp70 considerably postponed full-range activation of the NF-kB complex as related to the p65 transition to a cell nucleus. The delay was at least 6 h long; after that the complex recovered and the active period extended over the next 30–40 h (Fig. 2,5).

To explain this we had to assume the appearance of a factor (or factors) impeding NF-kB progression. We suspected the Hsp70 chaperone to be one of those factors, since its accumulation occurred during that period of time. Furthermore, known mediators of inflammation, LPS, interleukin-1β, nitric oxide and reactive oxygen species are able to induce a so-called heat shock response, including expression of stress proteins (Zhang et al 1994; Eizirik et al 1990; Malyshev et al 1995). Most convincing was the finding that heat shock or transfection with Hsp70 may strongly reduce the activity of NF-kB (Feinstein et al 1996). These data and the fact that mild heat shock stimulated the expression of Hsp70 and simultaneously hampered the uncovering of NF-kB activity in Molt4 cells led us to conclude that the chaperone can retard a part of the Rel protein in cytoplasm.

To reveal the physical association between the Hsp70 and proteins belonging to Rel family we employed an immunoprecipitation assay combined with Western blotting. No Hsp70 was found in co-immunoprecipitates obtained with the anti-p65, anti-c-Rel or anti-p50, nor the anti-p105 antibodies, probably due to the fact that the stress protein and the antibodies to those polypeptides possess closely located recognition sites. The interaction of p65, p50 and c-Rel with Hsp70 was clearly shown in the case, when the immunoprecipitation was carried out with the anti-Hsp70 monoclonal antibody (Fig. 4). Precursor of NF-kB (p105) and IkBα protein were not encountered in co-immunoprecipitates with the Hsp70 regardless of inducer type and whether or not the cells were heated. Analyzing the polypeptides that were not precipitated with the 3B5 antibody-Sepharose, we found that a certain amount of the Rel protein as well as that of the Hsp70 itself remained in an unbound state (data not shown). Hypothetically, the pool of Hsp70 includes the protein recognizable by the antibody and one that is inaccessible. The Rel protein pool includes the protein associated with both 'accessible' and 'inaccessible' Hsp70 and a part that is trapped by IkB.

Results presented in the paper raise several questions. The Rel protein-Hsp70 interaction was found in non-heated cells, in which the activation of NF-kB complex occurs efficiently. In our opinion this could be explained by low amounts of the chaperone not allowing it to suppress the NF-kB activation. Hsp70 content could be strongly augmented by a mild heat shock; when this happens the chaperone effectively captures members of the Rel family and retards them in cytoplasm. We suggest that Hsp70 or its constitutively expressed counterpart, Hsc70, both occurring in low amounts in non-stressed cells, are still able to recognize labile NF-kB complex; however, only heating or other Hsp70-inducing factors may trigger the elevation of Hsp70 content with a concomitant blocking of NF-kB activity. This new state lasts for rather a long time as indicated by the late revival of the p65 nuclear translocation. Destruction of the complex leads to a release of earlier 'hidden' molecules including p65 and other members of the Rel family.

Jäättelä and others showed that overexpression of Hsp70 in mouse fibrosarcoma cells WEHI-S stimulated by TNFα or UV-light did not influence the activity of NF-kB complex as shown by gel-shift analysis (Jäätelä 1993; Simon et al 1995). The discrepancy can be explained simply by a difference in cell type, in NF-kB inducers and in that, besides the p65, other members of the Rel family can contribute to reduced mobility of DNA probes in a

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gel-shift assay. On the other hand, and this appears more justified, in cells producing noticeable amounts of Hsp70, its binding to Rel family members already occurs, as in the untreated control cells in our case. Only increased accumulation of the chaperone may result in an substantial inhibition of NF-kB complex.

The results presented here may explain the growing data demonstrating that heat shock or transfection of cells by the Hsp70 gene negatively influences the activity of nitric oxide synthase (NOS) and TNFα (Bellmann et al 1996; Jäätelä 1993) both regulated by NF-kB (Baldwin 1996). Since Hsp70 binds Rel family members efficiently, it is clear why the attempts to lower the destructive effect of interleukin-1β on beta-cells by introducing Hsp70 gene or the protein itself were successful (Bellmann et al 1996; Margulis et al 1991). Using the other cell model, TNFα-mediated apoptosis in U-937 cells, we found that mild heat shock strongly reduced cytotoxic effect of the TNFα. As in the case of heat-shocked Molt4 cells Rel proteins occurred in complex with the Hsp70 (Guzhova and Margulis, unpublished data). Taken together these facts indicate that the protein might be a link in cell defenses against natural cytotoxins causing disfunction and ultimately death of various cells.

It appears most relevant that the delay of NF-kB complex activation almost exactly coincides with the period of time during which the cells persist with a high content of Hsp70 (Fig. 5) The protein was shown to bind only active members of the Rel family and not their precursor that possesses the inhibitory domain or the IkB protein itself (Fig. 4). The IkBα was shown to be proteolytically cleaved after PMA induction in heated and non-heated cells (Fig. 3). One could suggest that the Hsp70 is able to partially substitute for IkB. This assumption indicates a novel control over NF-kB activity. Hypothetically, being a regulatory factor, the Hsp70/Hsc70 chaperone, would not respond to signals that are known to be mediated by PKC or ceramide-sphingomyelin pathways (Baeuerle and Henkel 1994). Only time (Fig. 5) or factors inhibiting the heat shock response, are able to activate Rel proteins and trigger systems governed by the NF-kB complex.

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