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Effects of heat shock on gene expression and subcellular protein distribution in Chinese hamster ovary cells

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ABSTRACT

Incubation of Chinese Hamster Ovary (CHO) cells for one hour at 43°C results in several obvious changes in protein distribution and protein synthesis. One major protein of the cytoplasm (molecular weight 45,000 daltons), also present as a minor component in the nucleus, rapidly disappeared while several proteins, especially high molecular weight peptides, were induced by heat shock. Localization of the proteins in the cytoplasm, extranucleolar chromatin and nucleolar bodies has been carried out. Different sets of induced proteins appear in each subcellular compartment. Four hours after restoration of the normal temperature, the normal pattern of protein synthesis was observed. The 45,000 dalton protein reappeared first. Relations between structural and functional alterations and changes in protein distribution are suggested.

INTRODUCTION

Regulation of cellular macromolecular synthesis has been studied through perturbations induced by metabolic analogues (1) or by abnormal growth conditions, such as altering the temperature of incubation (1-3). Several studies on Drosophila melanogaster cells exposed to 37°C have demonstrated relations between the activation of a series of specific genes, the appearance of new puffs, and the induction of the synthesis of a small number of proteins whereas the synthesis of most cellular proteins made at 25°C was strongly reduced (4-7). Induction of the synthesis of new proteins by incubation at supranormal temperatures has also been reported in other systems (1).

The nucleolus of eukaryotic cells has been shown to be a particularly sensitive target to effects of supranormal temperatures. Ultrastructural modifications of intranucleolar chromatin and inhibition of RNA synthesis are first observed, followed by a general condensation of nuclear chromatin (8, 9). These alterations could result from the appearance or disappearance of proteins in specific subcellular fractions.

The results presented in this paper clearly show that in Chinese Hamster Ovary (CHO) cells exposed to supranormal temperature (43°C), the synthesis of new proteins that are specific for different subcellular compartments is induced. The induction of these proteins continues during at least the first hour following the heat shock in cells returned to 37°C.

MATERIALS AND METHODS

Chinese Hamster Ovary cells were grown as previously described (10, 11). In the heat shock experiments, Falcon culture flasks were immersed for 1 hour in a water bath maintained at 43°C. Prior to labeling, cells were incubated for 15 min in Eagle's MEM 011 (Eurobio) minus methionine. Omission of this amino acid did not induce observable changes in cell growth for the short periods we have studied. Cells were labeled for 1 hour in Eagle's MEM 011 minus methionine containing 4 µCi/ml of ^{35}S-methionine (1005 Ci/mM, Amersham).

Cell fractionation : Cells in monolayer were immediately detached with glass beads (Ø 0.25 - 0.30 mm ; B. Braun Melsungen) in R buffer (40 mM Tris-HCl pH 7.7, 25 mM KCl, 4 mM $MgCl_2$ and 10 µg/ml polyvinylsulphate) in the presence of 0.5 % (v/v) cemulsol NPT 6,500 µg/ml collagenase and proteolytic inhibitors (1 mM phenyl methyl sulfonylfluoride and 1 mM diisopropylfluorophosphate) that were also added to all buffers. After 2 min handshaking in the cold (4°C), glass beads were discarded by decantation and the suspension containing detached cells was submitted to the action of an Ultra-Turrax to prepare nuclei as previously described (12).

Purified nuclei were resuspended in buffer A (2.1 % Ficoll, 10 mM Tris-HCl pH 7.4, 5.5 mM $MgCl_2$, 0.25 mM 2-mercaptoethanol, 10 µg/ml polyvinyl sulfate). The nuclear suspension was then submitted to a first sonication cycle (60 to 100 s, by 10 s waves with 20 s rest inbetween) with the Raytheon sonic oscillator (10 kHz, 200 W). Mg^{2+} concentration was then lowered to 3 mM, by addition of an equal volume of buffer B (identical to A except for 0.5 mM $MgCl_2$) and an additional sonication step was carried out in order to achieve the dispersion of most extra-nucleolar chromatin aggregates while the nucleolar structures were preserved. Nucleoli were recovered from the sonicated suspension by a 5 min centrifugation at 500 x*g*, resuspended in buffer C (10 mM Tris-HCl pH 7.4, 20 mM KCl, 2 mM $MgCl_2$, 20 µg/ml polyvinylsulfate) and collected by a 5 min centrifugation at 1500 x*g*. The two supernatants were mixed and represented extranucleolar chromatin.

Nucleolar fractionation : Nucleolar pellets were resuspended by pipetting in 1 ml phosphate buffer (0.7 mM potassium phosphate, pH 6.8) containing 2 mM Mg^{2+}. The suspension was allowed to stand for 30 min at 0—4°C before being submitted to a 10 s sonication (Branson 122 Ultrasonifier with microtip, 20 kHz, 100 W). The suspension was then centrifuged for 5 min at 1500 x*g*. The resulting pellet (nucleolar bodies) contained the bulk of rDNA and nucleolar RNPs. The supernatant contained essentially perinucleolar chromatin (11).

Analysis of proteins by slab gel electrophoresis : Protein samples were prepared and analysed by electrophoresis on 10-16 % polyacrylamide gradient slab gel. The gradient of 10-16 % w/v acrylamide, 0.13-0.21 % w/v bisacrymide, was performed with an autodensiflow Büchler in the following buffer : 0.4 M Tris-HCl pH 8.8, 0.1 % SDS, 0.1 mM potassium ferricyanure. Polyacrylamide gels were processed for fluorography and exposed to KODIREX autoprocessing (13).

RESULTS AND DISCUSSION

Effects of heat shock on major protein distribution among different subcellular compartments : In preliminary experiments we have observed that crucial changes in nuclear structure and RNA biosynthesis occurred in CHO cells incubated one hour at 43°C. The effects were completely reversed 10 hours after the heat shock in cells returned to 37°C.

In an attempt to correlate the ultrastructural and functional alterations with changes in protein distribution, we have analysed in parallel proteins extracted from different subcellular compartments of control and heat shocked cells. Proteins from cytoplasm (lanes 1, 2), extranucleolar chromatin (lanes 3, 4) and nucleolar bodies (lanes 5, 6) were partially resolved by SDS polyacrylamide gel electrophoresis and Coomassie blue staining of the electrophoregrams (Fig.1). Nucleolar bodies were prepared from nucleoli by removal of perinucleolar chromatin and were used in this study since we have demonstrated (12) that perinucleolar chromatin (70 % of nucleolar DNA) has a protein composition similar to extranucleolar chromatin while specific nucleolar species are present in nucleolar bodies. In comparison to control cells (lanes 2, 4, 6), incubation of cells one hour at 43°C (lanes 1, 3, 5) results in several changes in protein distribution which are specific to the different cellular subfractions. In the cytoplasm, the most obvious modifications were the disappearance of two major proteins (45K and 105K) while several new minor bands with molecular weights higher than

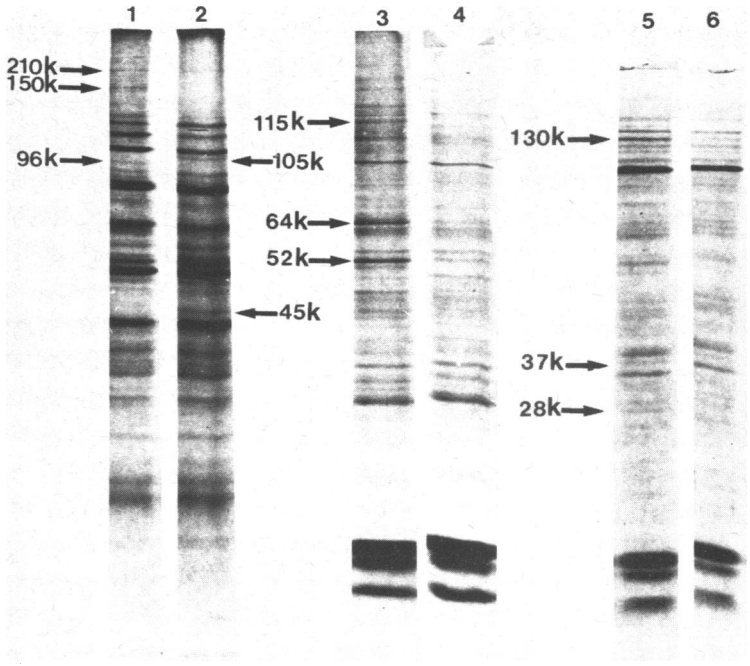

Fig. 1 : Gel electrophoresis of proteins isolated from cytoplasm, extranucleolar chromatin, nucleolar bodies. Extranucleolar chromatin was obtained after spinning down residual nucleolar fragments for 5 min at 2 500 x*g*. Nucleolar bodies were isolated as described in Materials and Methods. Proteins were prepared and electrophoresed on 10-16 % linear sodium dodecylsulfate polyacrylamide slab gels. After electrophoresis, gels were stained with Coomassie blue. Proteins isolated from : slots 1-2, cytoplasm ; slots 3-4, extranucleolar chromatin ; slots 5-6, nucleolar bodies. Slots 1, 3, 5 : proteins from heat shock cells (1 h at 43°C) ; slots 2, 4, 6 : proteins from control cells. Arrows point out proteins which presented the most obvious changes between control and heat shock cells. Molecular weights have been determined using markers in parallel.

96K appeared. In the two nuclear subfractions, no disappearance of major proteins was observed. On the other hand, in the extranucleolar chromatin a large accumulation of two bands (52K and 64K) occurred and several new and minor, high molecular weight bands were induced by the heat shock while in the nucleolar body only two bands, 28K and 130K, were induced.

Effects of heat shock on protein labeling with ^{35}*S-methionine :* In the analytical system used, Coomassie blue staining allowed the observation of

TABLE I : Effect of heat shock on the levels of synthesis of cytoplasmic proteins and on their transport to extra- and intranucleolar compartments

	37°C	T_0	T_1	T_4
Cytoplasm	18	37	1.2	3.4
Nucleolar body	0.32	0.43	0.03	0.03
Extranucleolar chromatin	3.8	7.3	0.5	0.8

Cells were heat shocked for 1 h at 43°C (see Materials and Methods) and labeled with ^{35}S-methionine 1 h at 43°C (T_0), 1 h at 37°C just after heat shock (T_1) or 4 hours after heat shock (T_4). Cpm x $10^{-6}/10^7$ cells.

major component variations while induction of the synthesis of new proteins present only in small amounts was not detected. In a second set of experiments, biosynthesis of proteins and their transport into nuclei and nucleoli were followed in cells incubated one hour with ^{35}S-methionine at 43°C or after heat shock at 37°C. Since methionine was not evenly distributed through proteins, similar experiments were carried out using ^{3}H - leucine and lead to similar quantitative results.

As is shown in Table I, total incorporation of ^{35}S-methionine was two times enhanced in cells labeled for one hour at 43°C. This result corresponds to the superposition of two phenomena : during the first 5 minutes at 43°C until an equilibration of medium temperature was reached, a high increase in protein synthesis occurred, then, the rate of ^{35}S-methionine incorporation fell to less than 10 % of control. Levels of incorporation at 37°C just after or 4 hours after heat shock were respectively 8 % and 20 % of control and the repartition of labeled proteins between nucleolar body, extranucleolar chromatin and cytoplasm was not significantly modified.

In cells labeled one hour at 43°C and, more clearly, in heat shocked cells labeled one hour at 37°C, proteins specific to different subcellular compartments appeared while others were no longer synthesized. We have first verified through "chase experiments" that most of the one hour labeled proteins recovered in the cytoplasm are not later transferred to the nucleus.

Cytoplasmic proteins synthesized after a heat shock : CHO cells were labeled for one hour with ^{35}S-methionine, either at 43°C (lane 1) or at 37°C, immediately (lanes 2, 2') or 4 hours (lanes 3, 3') after a return to normal growth conditions. In the cytoplasmic fraction (Fig.2), two proteins were induced at 43°C with molecular weight 64,000 and 60,000 daltons, while a

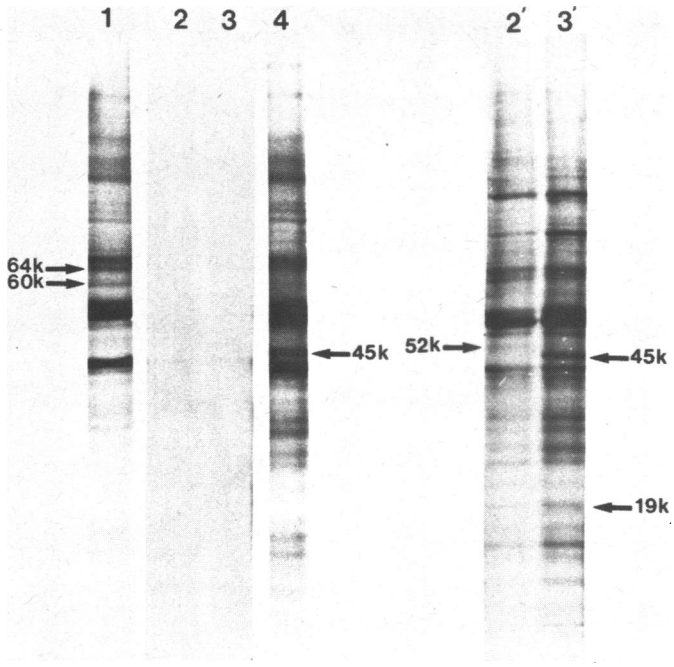

Fig. 2 : Fluorogram of a sodium dodecyl sulfate polyacrylamide slab gel of cytoplasmic ^{35}S-proteins extracted from CHO cells incubated 1 h at 43°C. Cells were labeled 1 h (see Materials and Methods) at 43°C (lane 1), just after heat shock (lanes 2, 2') and 4 h after heat shock (lanes 3, 3'). Lane 4 : control cells labeled 1 h at 37°C. Lanes 1-4 correspond to 24 h exposure at -80°C. Lanes 2' and 3' correspond to 168 h exposure at -80°C.

protein with a molecular weight of 45,000 daltons was no longer present. Three induced bands (96K, 150K, 210K) were detected by Coomassie blue staining of the electrophoregrams and not on the autoradiograms and could result from an absence of methionine in the corresponding peptides.

In cells labeled one hour at 37°C, immediately after heat shock, the same pattern of proteins was induced with an additional protein with a 52,000 dalton molecular weight. Total protein synthesis being only 8 % of control (Table I), no labeled proteins appeared on the fluorogram after 24 h exposure of the gel (lanes 2, 3) but were visualised after 168 hours (lanes 2', 3'). Furthermore, the relative amount of some peptides (i.e., molecular weight 19,000 daltons) was markedly increased and could correspond to a relatively higher level of biosynthesis of preexisting species or to the

induction of peptides not resolved in our analytical system. Four hours after a return to normal growth conditions, the pattern of cytoplasmic cellular protein synthesis was normal (lane 4) : the 45K protein was again synthesized while the 52K protein was no longer present. Though the total capacity of protein biosynthesis was reduced, the qualitative global pattern was only slightly modified. The characterization of 45K protein whose disappearance is the first event during the heat shock and reappearance the first event during the recovery at 37°C is now under investigation.

Nuclear proteins synthesized after heat shock : The pattern of extranucleolar chromatin proteins, synthesized during or after heat shock, was markedly different from control cells (Fig. 3). Two minor bands, 25K and 45K, disappeared and several new bands appeared. The most obvious changes were the induction of bands 50K, 64K, 66K and several high molecular weight bands among which are 105K, 130K and 150K. In addition, the bands 42K, 52K

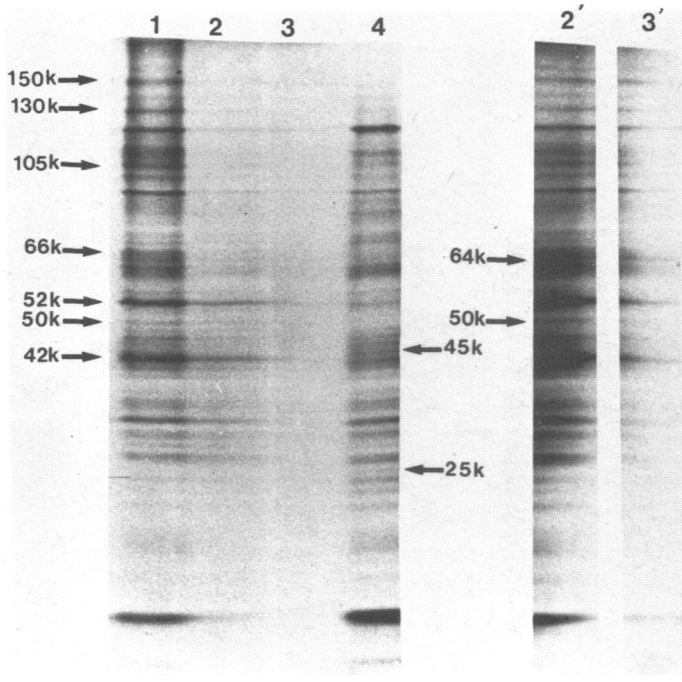

Fig. 3 : Fluorogram of a sodium dodecyl sulfate polyacrylamide slab gel of extranucleolar ^{35}S-proteins extracted from CHO cells incubated 1 h at 43°C. Legend as described in figures 1 and 2.

and 66K, that were present in slight amount in control cells, became major components. The same patterns were obtained in heat shocked cells incubated at 37°C with ^{35}S-methionine one hour or 4 hours after treatment.

The accumulation of high molecular weight proteins could represent induced peptides involved in chromatin condensation. Alternatively, peptides could enter the nucleus in the form of large precursors which are then cleaved, this step not being restored during the four hours after return to normal growth conditions. This result may be related to data obtained by Van Zaane et al. (14, 15) that show a large accumulation of high molecular weight precursors for animal virus structural proteins when canavanine, an arginine analogue, was added to the cell culture medium.

In the nucleolar body, similar modifications in the pattern of newly synthesized proteins were observed (Fig.4). Several high molecular weight bands were induced and one of them (125K) appeared to be specific for this fraction. In addition, a 70K molecular weight protein accumulated during heat shock and a 52K band was induced. A correlation between the

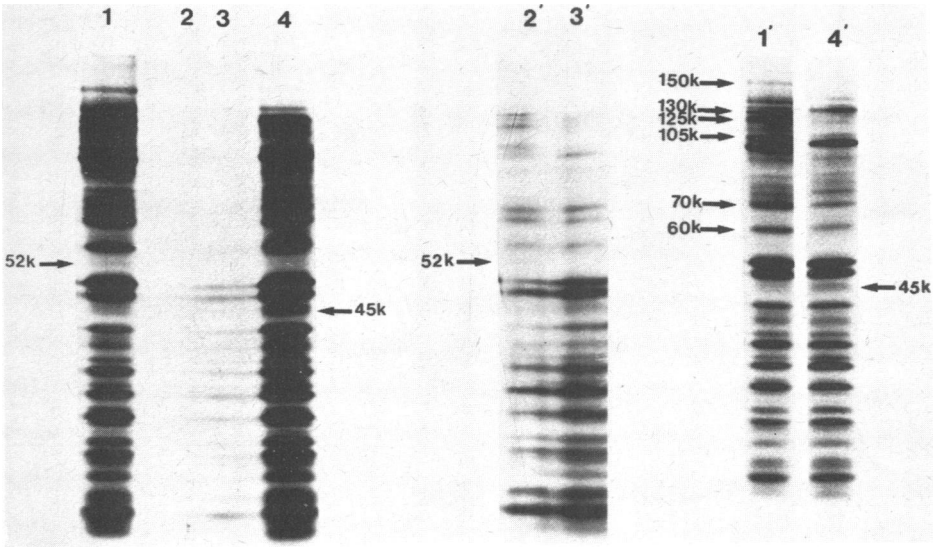

Fig. 4 : Fluorogram of a sodium dodecyl sulfate polyacrylamide slab gel of nucleolar bodies ^{35}S-proteins extracted from CHO cells incubated 1 h at 43°C. Legends as described in figures 1 and 2. Lanes 1' and 4' correspond to 10 h exposure at -80°C.

presence of these high molecular weight peptides and the previously observed condensation of intranucleolar chromatin (8) is suggested.

Finally the several proteins induced in CHO cells could not easily be related to results presented by Kelley and Schlessinger on baby hamster kidney cells in which superposition of total cellular proteins did not allow a clear resolution of different species (1).

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REFERENCES

1. KELLEY, P.M. and SCHLESSINGER, M.J. (1978) Cell, 15, 1277-1286.
2. TISSIERES, A., MITCHELL, H.K. and TRACY, V.M. (1974), J.Mol. Biol. 84, 389-398.
3. LEWIS, M., HELMSING, P.J. and ASHBURNER, M. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 3604-3608
4. CRAIG, E.A., McCARTHY, B.J. and WADSWORTH, S.C. (1979) Cell, 16, 575-588.
5. LIS, J.T., PRESTIDGE, L. and HOGNESS, D.S. (1978) Cell, 14, 901-919.
6. WU, C., BINGHAM, P.M., LIVAK, K.J., HOLMGRIN, R. and ELGIN, S.C.R. (1979) Cell, 16, 797-806.
7. WU, C., WONG, Y.C. and ELGIN, S.C.R. (1979) Cell, 16, 807-814.
8. SIMARD, R., AMALRIC, F. and ZALTA, J.P. (1969) Exp Cell Res. 55, 359-369.
9. AMALRIC, F., ZALTA, J.P. and SIMARD, R. (1969) Exp. Cell. Res. 55, 370-376.
10. CABOCHE, M. and BACHELLERIE, J.P. (1977) Eur. J. Biochem. 74, 19-29.
11. BACHELLERIE, J.P., NICOLOSO, M. and ZALTA, J.P. (1977) Eur. J. Biochem. 79, 23-32.
12. ZALTA, J. and ZALTA, J.P. (1973) Methods in Cell Biology, Prescott, D.M., ed., Vol. 6, pp. 317-324, Academic Press, New-York.
13. BONNER, W.N. and LASKEY, R.A. (1974) Eur. J. Biochem. 46, 83-88.
14. VAN ZAANE, D., GIELKENS, A.L.J., DEKKER-MICHIELSEN, M.J.A. and BLOEMERS, H.P.J. (1975) Virology, 67, 544-552.
15. VAN ZAANE, D., DEKKER-MICHIELSEN, M.J.A. and BLOEMERS, H.P.J. (1976), Virology, 75, 113-129.