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INDUCTION OF NEW PROTEINS IN THE NUCLEAR MATRIX OF CHO CELLS BY A HEAT SHOCK : DETECTION OF A SPECIFIC SET IN THE NUCLEOLAR MATRIX

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An early response to an incubation of CHO cells for one hour at 43°C is the appearance in the nuclear matrix of new proteins : 2 proteins (48 and 46 kDa) were associated with the extranucleolar part of the nuclear matrix, 6 other proteins (130, 100, 95, 85, 76 and 70 kDa) were present in matrices prepared from isolated nucleoli. In addition, a large increase in the amount of associated minor components is observed. It is suggested that these early modifications in composition of the matrix are related to the inhibition of rRNA and hnRNA synthesis and maturation.

Substantial evidence points to the existence of an underlying protein matrix in the eukaryotic and prokaryotic cell nucleus (1-6). The nuclear matrix consists of a group of residual, non-histone proteins which remain after extensive extraction of isolated nuclei by high salt, nucleases, and detergent. Electron microscopy identifies three components of this scaffold-like structure : the pore complex-lamina layer, situated along the inner nuclear membrane and characterized by three major polypeptides (2, 3), the residual nucleoli, which maintain an identifiable nucleolar form (4), and the intranuclear network, a loose reticulum interconnecting interior regions of the nucleus (5).

A variety of metabolic and structural roles has been attributed to the nuclear matrix. In particular, its implication in hnRNA transcription, processing and transport has been suggested (6, 7), but only a few correlations have been established between protein composition of the nuclear matrix and nuclear RNA metabolism (8, 9). None of these studies, however, was able to discriminate among the different components of the matrix.

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We have developed an experimental heat shock system in which incubation of CHO cells for one hour at 43°C is shown to induce a reversible inhibition of rRNA and hnRNA synthesis and maturation (10, 11). In the present report, we have used this heat shock system to analyze and compare the protein composition of the nuclear and the nucleolar matrices, under normal physiological conditions and under conditions known to arrest nuclear RNA transcription and processing. Heat shock induces major quantitative and qualitative changes in matrix proteins. Some of these changes are specific for one or another of the nuclear compartments.

MATERIALS AND METHODS

Chinese hamster ovary cells (CHO) were grown in monolayer culture at 37°C in Falcon flasks. When a large volume of cells was required for the isolation of nucleoli from control and heat shocked cells, suspension cultures were used (12). For a heat shock, culture flasks were immersed for 1 h in a water bath maintained at 43°C. The temperature inside the flasks was equilibrated in less than 5 min (10).

Cell fractionation was carried out according to Bachellerie et al. (12) in the presence of $PhMeSO_2F$ and leupeptin at 4°C. Nucleoli were prepared from purified nuclei by two sonication cycles, the first in the presence of 5.5 mM $MgCl_2$ followed by one in 3 mM $MgCl_2$. Isolation of the nucleolar fraction was carried out by differential centrifugation (13). Matrices were prepared from isolated nuclei and from purified nucleoli essentially as described by Long et al. (14), in the presence of $PhhMeSO_2F$ and leupeptin. An additional RNase treatment (100 ug A + 1000 units T_1/ml) for 15 min at 30°C was included. For nucleolar matrix preparation, the first centrifugations were carried out at 1500 g for 10 min in place of 500 g.

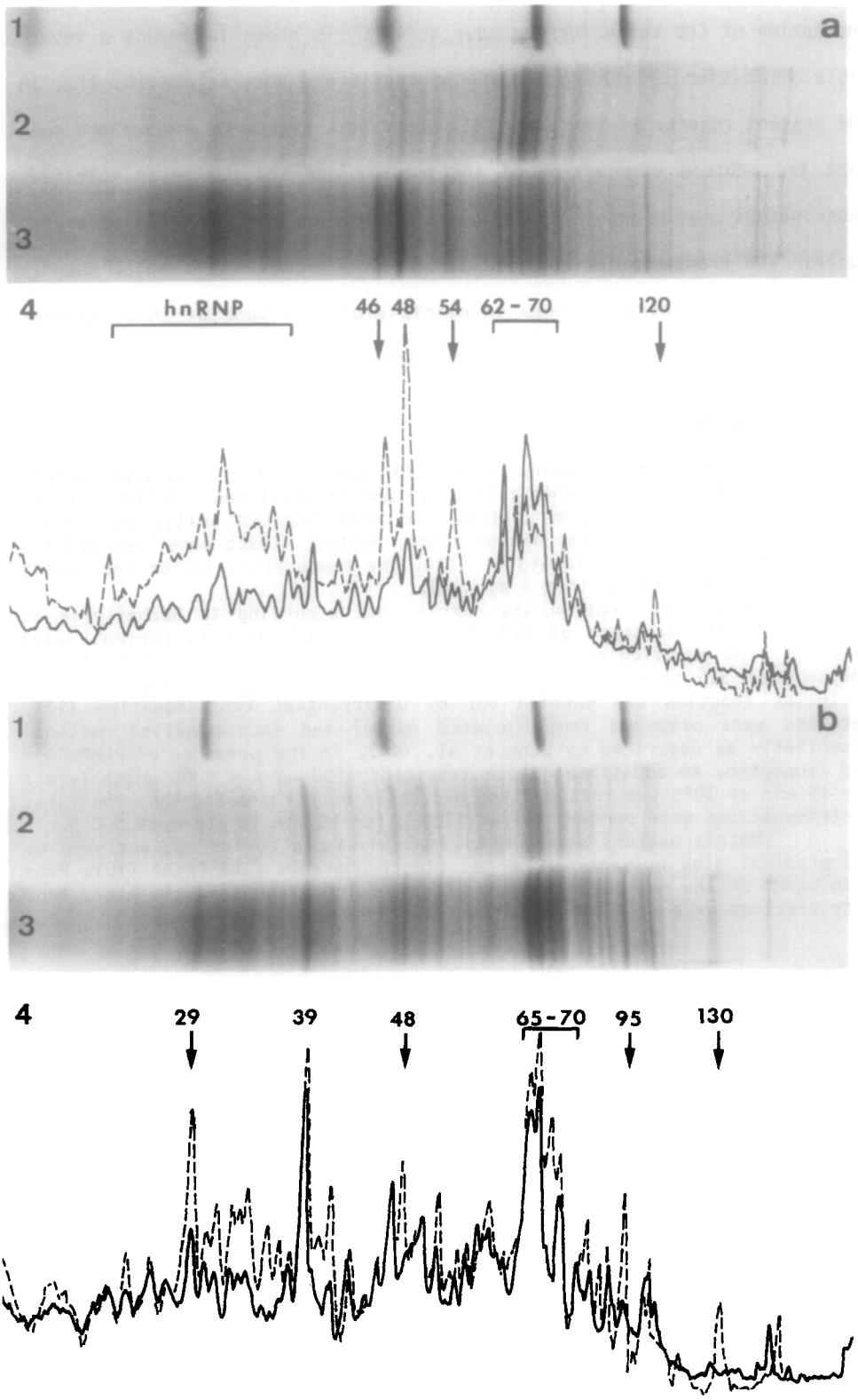
Matrix pellets were solubilized in sample buffer and analyzed by SDS-gradient slab polyacrylamide gel electrophoresis (SDS-PAGE) (10). Protein bands on the gels were detected by staining with Coomassie blue. Density tracings were made with a Vernon microdensimeter.

RESULTS

Protein composition of nuclear and nucleolar matrices

Nuclear matrix preparations from CHO cells display residual nuclei containing the typical morphological components. Nucleolar matrices prepared from isolated nucleoli have a structure nearly identical to that of the residual nucleoli in the nuclear matrix, as observed by electron microscopy (15).

SDS-PAGE analysis of the nuclear matrix shows major protein bands which migrate with apparent molecular weights between 62 and 68 kDa, and a number of minor components (fig. 1a). Among the major species are the three


1
2
3
a
4
hnRNP
46
48
54
62 - 70
120
1
2
3
b
4
29
39
48
65 - 70
95
130

polypeptides of the lamina (2, 3). In the nucleolar matrices, four proteins are present in large amounts : 72, 67, 65 and 39 kDa. Several other prominent bands are also detected (fig. 1b).

From the purity of our nucleolar fractions and from our ability to reproduce these results, we are able to state that a residual protein matrix composed of a unique group of polypeptides is present in the nucleolus. We have not been able to determine the quantitative significance of these nucleolar matrix polypeptides in our preparations of total nuclear matrix proteins. It is probable, however, that, except for the lamina bands, much of the protein content of the nuclear matrix is essentially derived from the residual nucleolar matrix.

Effect of heat shock on nuclear and nucleolar matrix composition

A number of significant modifications in the matrix protein composition after heat shock are found. The most striking of these is the overall increase in the recovered protein content for the same number of cells (figs. 1a and b). In the nuclear matrix, new polypeptides not present in controls or present in low amounts are induced. They have molecular weights of 120, 95, 74, 70, 54, 48 and 46 kDa. A net increase in peptides between 30 and 40 kDa, the range of the structural hnRNP proteins, is clearly observed. A decrease in the amount of the 66 kDa lamina band is also noted immediately after heat shock (fig. 1a).

A comparison of nuclear and nucleolar matrix composition allows us to localize the new proteins. The interesting result is that some of the induced proteins are exclusively present in one or the other fraction.

Figure 1 : Analysis of protein composition of nuclear (a) and nucleolar (b) matrices by 10-16 % polyacrylamide gradient gel electrophoresis.

Control cells at 37°C (2 and 4 ——) : Cells submitted to a 1 h heat shock at 43°C (3 and 4 -----) a : nuclear matrices - b : nucleolar matrices. Each sample contains matrix proteins obtained from 2.10^7 cells. Scanning of the Coomassie blue stained gels was carried out independently for each lane after integration of total proteins. The height of a given peak gives the relative percentage of the species in the sample. A comparison of the heights of two peaks from separate lanes is thus not directly related to its amount in each sample. Molecular weight standards (1) : 95,000, 67,000, 43,000, 30,000 and 20,000.

Prominent new proteins with molecular weights of 130, 100, 95, 85, 76 and 70 kDa are specifically present in the nucleolar matrix, while others (48 and 46 kDa) are only present in the extranucleolar part of nuclear matrices. The relative proportion of nucleolar matrix proteins in the total nuclear matrix preparations after heat shock is much lower than in the control, in large part due to the dramatic increase in the hnRNP-like proteins.

DISCUSSION

The numerous studies of the heat shock response in eukaryotic and procaryotic organisms have dealt principally with the long term effects; these studies have well documented the appearance of a small set of induced proteins, the heat shock proteins (hsps), and the transcription of the hsp mRNAs, events generally taking place after several hours at the elevated temperature (reviews in 16 and 17). Data on the intracellular distribution of the heat shock proteins reveal that some of them accumulate in the cell nucleus (18-21) and that at least two heat shock proteins (70 and 34 kDa) are associated with the nuclear skeleton (20, 21).

In CHO cells, one of the early effects of a heat shock is the inhibition of rRNA and hnRNA synthesis and processing (11, 22). The present results demonstrate that, in addition, two sets of early proteins appear at the nuclear matrix level : one in the nucleolar matrix and the other in the extranucleolar part of the nucleus. We cannot yet resolve the question of the equivalence between some of the early matrix proteins in CHO cells and the matrix-associated heat shock proteins.

The presumptive role(s) of the new matrix proteins in the early response to heat shock in CHO cells remains to be demonstrated. One possibility is that they are implicated in the regulation of rRNA and hnRNA synthesis and processing. In this direction, we have recently shown (in preparation) that several of the induced nucleolar proteins are immunologically related to a major nucleolar protein (100 kDa), a component of preribosomes, which appears to be involved in the metabolism of pre-rRNA

(23, 24). Concomitant with the early block in pre rRNA processing after incubation at 43°C, preribosomes become poorly extractable from isolated nucleoli : 15 % of nucleolar ribonucleoproteins were recovered in the soluble fraction instead of 70 % in controls (22). These results taken together suggest that an immediate effect of a heat shock would be to fix preribosomes in place on the nucleolar matrix. In the exponentially growing cell, preribosomes are only transitorily associated with this matrix and proteins involved in their metabolism are thus present in such a low amount that they are undetectable. By extension, the increase in hnRNP-like proteins after heat shock could result from the fixation of hnRNP particles on the nuclear matrix concomitant with the inhibition of hnRNA processing.

As this manuscript was completed, a paper by Reiter and Penman (9) reported the appearance in the nuclear matrix during heat shock of "prompt" proteins that look to be identical to the species described in our report.

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