PHOSPHORYLATED PROTEINS INVOLVED IN THE REGULATION OF rRNA SYNTHESIS IN CHO CELLS RECOVERING FROM HEAT SHOCK

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1. Introduction

Various hypothesis have been advanced to explain the regulation of rRNA synthesis. Transcription of ribosomal genes may be dependent on the presence of rapidly turning over proteins, on the concentration of amino acids [1–5], or under the control of intracellular ATP and GTP pool sizes [6]. Also, there may be a role for protein phosphorylation in the regulation of gene expression [7–10]. Finally, interrelations between these various control mechanisms must also be considered.

We have investigated the role of a number of factors postulated to be involved in the regulation of transcription in a heat shock system using CHO cells. Incubation of cells for 1 h at 43°C induces reversible inhibition of rRNA synthesis [11–12]. Following heat shock, cells incubated at 37°C recover a normal level of RNA synthesis by 7 h. Results indicate that in our experimental system, RNA polymerase activities ATP and GTP pool concentrations and overall proteins transport were not directly related to rRNA synthesis. On the other hand, heat shock induced the phosphorylation of two nuclear proteins, one of which is specific to the nucleolus (95 000 M_r). An apparent correlation between the initiation of RNA synthesis and the dephosphorylation of the 95 000 M_r protein was observed.

2. Materials and methods

Chinese Hamster ovary cells (CHO) were grown and heat shock experiments were carried out as in [12]. After heat shock (1 h at 43°C), cells were allowed to recover at 37°C. Cells were collected [12] and fractionated into nuclei and nucleoli according to [13]. RNA polymerases were extracted from purified nuclei by high salt sonication [14]. The assays were done as in [15]. The relative concentrations of ATP, GTP and UTP were determined by chromatography on polyethyleneimine (PEI) thin-layer plates. ^{32}P-Labeled cell pellets (10^7 cells) were treated by 15% formic acid for 30 min at 4°C under vigorous agitation [6]. Aliquots of 10 μl of the extracts were analyzed by polyethyleneimine chromatography and the plates were developed with 1.5 M KH_2PO_4 (pH 3.5). The chromatograms were cut, nucleotides were eluted with 15% formic acid and the radioactivity measured. A_{260} of the eluted ATP was read. Analysis of proteins was carried out by slab-gel electrophoresis as in [12].

3. Results

3.1. *RNA synthesis after heat shock*

Incubation of CHO cells for 1 h at 43°C induces a reversible inhibition of high molecular weight RNA synthesis. In the first 6 h following heat shock, no RNA synthesis was detected in cells incubated at 37°C. RNA was extracted from isolated nucleoli and from the non-nucleolar fraction of the nucleus. More than 90% of the RNA contained in the nucleolar fraction was preribosomal RNAs while hnRNAs were present in the non-nucleolar fraction. As shown in fig.1, synthesis of hnRNA and rRNA both resumed after 8 h recovery at 37°C. By 10 h after heat shock the level of rRNA synthesis was twice that of the control and then returned to control level by 14 h. This system appeared to be particularly suitable to study the

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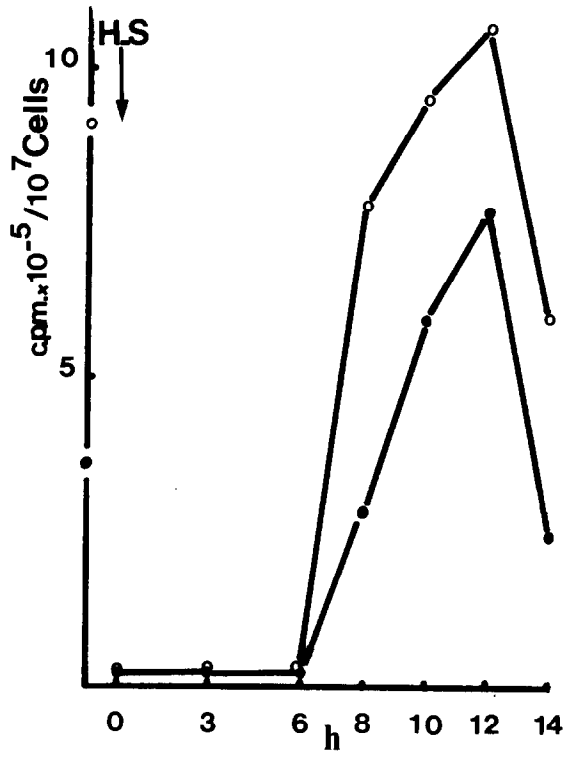

Fig.1. RNA synthesis in CHO cells recovering from a heat shock. Cells were heat-shocked for 1 h at 43°C, and allowed to recover at 37°C. At various times, aliquots were incubated for 15 min in the presence of [^{3}H]U (1 μCi/ml, 20 Ci/mmol). RNA was extracted from purified nucleoli (•—•) and from the non-nucleolar fraction of the nucleus (○—○) and assayed for incorporated ^{3}H radioactivity.

various factors involved in the regulation of rRNA synthesis.

3.2. *RNA polymerase activities after heat shock*

RNA polymerases were solubilized from heat shocked and control cells. Activities of RNA polymerases A and B were determined in the presence or the absence of α amanitin, respectively. During the first hours following heat shock, a decrease of 20% was observed for both activities, followed by an enhancement of 50–100% after 10 h recovery (table 1). Consequently, the complete inhibition of RNA synthesis could not result simply from the inactivation of RNA polymerases. The increase in RNA synthesis during recovery, however, followed the enhancement of polymerase activities.

3.3. *Variation in ATP and GTP pools of heat-shocked cells during recovery*

The possible influence of the pool sizes of nucleotide triphosphates on RNA synthesis was examined in cells recovering at 37°C from a heat shock. The pool size of ATP was directly measured by the luciferin–luciferase technique [16] (table 2). Slight variations of the ATP pool were observed during or after heat shock. This finding demonstrates that transcription of rRNA was not only under control of the nucleoside triphosphate levels in the cell. After 4 h recovery, rRNA synthesis had not resumed in heat-shocked cells while the ATP pool size was identical to that of control cells. On the contrary, after 7 h when RNA synthesis resumed, a decrease in the ATP pool occured. Similar variations of the GTP pool were observed, while the UTP pool remained constant throughout the experiment (table 2).

3.4. *Phosphorylated protein content of heat-shocked cells during recovery*

As reported [12], heat shock-induced discrete variations in nucleolar protein content and especially the appearance of new species. After 4 h recovery at

Table 1
RNA polymerase activity in cells recovering from a heat shock

Recovery time (h)	Total activity (A + B) (%)	RNA polymerase A activity (%)	'In vivo' RNA synthesis (%)
Control	100	48	100
4	64 ± 5	32 ± 4	8 ± 10
10	160 ± 30	75 ± 10	140 ± 14

Cells were heat shocked and allowed to recover at 37°C as in fig.1; activities were measured on extracts from purified nuclei as in [15]. RNA polymerase A activity was determined in the presence of 100 μg α-aminitin/ml. Results are expressed as % of activity determined on the same number of control cells. Percentages of 'in vivo' RNA synthesis were calculated from fig.1

Table 2
ATP pool in cells recovering from a heat shock

Recovery time (h)	$\mu M/10^7$ cells	Spec. act. (cpm . $10^{-4}/\mu M$)	GTP (%)	UTP (%)
Control	160 ± 10	0.11	100	100
1	164 ± 10	0.03	124 ± 15	102 ± 15
4	145 ± 10	0.02	100 ± 15	95 ± 15
8	29 ± 6	0.10	52 ± 10	72 ± 13

The pool size of ATP was measured by the luciferin–luciferase technique [16]. To determine ATP specific activity, heat-shocked cells (1 h at 43°C) were incubated appropriate times at 37°C and labeled for 1 h with [^{32}P]orthophosphate. ATP, GTP and UTP were isolated by chromatography on polyethyleneimine thin-layer plate. For GTP and UTP pool determination, cells were ^{32}P-labeled for a long time (15 h), heat shocked for 1 h at 43°C and then allowed to recover at 37°C. Results are expressed as % of control cells harvested before heat shock

37°C, newly synthetized protein appeared in the nucleus and the necleolus, showing the same distribution pattern as in control cells. However, although a large decrease in total protein phosphorylation occurred (80%) only 3 changes were observed in the pattern of phosphorylated proteins: the phosphorylation of a 95 000 M_r nucleolar protein and a 54 000 M_r non-nucleolar nuclear protein and the dephosphorylation of a 35 000 M_r non-nucleolar nuclear protein [17]. These changes were still observed during the first 6 h at 37°C following heat shock. On the other hand, after 7 h when RNA synthesis had resumed a normal phosphorylation pattern was observed (fig.2).

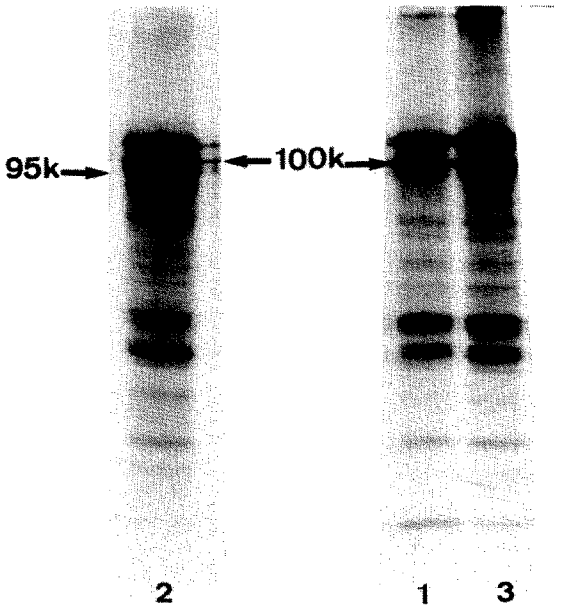

Fig.2. Detection of phosphorylated protein from control and heat-shocked cells by chromatography on slab-gel electrophoresis: CHO cells were ^{32}P labeled for 1 h at 37°C (1) or at 43°C (2) or at 37°C 1 h after heat shock [3]. Nucleoli were prepared [13] and proteins analysed by electrophoresis on 10–16% gradient slab gels [12].

4. Conclusion

In the experimental system described here, rRNA synthesis was successively turned off and turned on again. Examination of the various factors supposed to be involved in the regulation of rRNA synthesis showed them to be differently affected by incubation at supranormal temperature. The crucial point is that after 4 h recovery at 37°C, new RNA was synthesized while most of the factors considered were present in concentrations almost identical to those in control cells. Thus, the only apparent correlation was that between rRNA synthesis induction and the dephosphorylation of two specific proteins. These results are relevant to the argument that protein phosphorylation plays a key role in the regulation of gene expression [7–10].

The effect of the phosphorylated or dephosphorylated 95 000 M_r nucleolar protein on 'in vitro' transcription of cloned rDNA is now under investigation.

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References

[1] Holley, R. W. and Kiernan, J. A. (1974) Proc. Natl. Acad. Sci. USA 71, 2942–2945.
[2] Muramatsa, M., Shimada, N. and Higashinakagarva, T. (1970) J. Mol. Biol. 53, 91–106.
[3] Yu, F. L. and Feilgelson, P. (1972) Proc. Natl. Acad. Sci. USA 69, 2833–2837.
[4] Chesterton, C. J., Coupar, B. E. H., Butterworth, P. M. W., Buss, J. and Green, M. H. (1975) Eur. J. Biochem. 57, 79–84.
[5] Lampert, A. and Feilgelson, P. (1974) Biochem. Biophys. Res. Commun. 58, 1030–1038.
[6] Grummt, I. and Grummt, G. (1976) Cell 7, 447–453.
[7] Kleinsmith, L. J. (1975) J. Cell. Physiol. 85, 459–475.
[8] Elgin, S. C. R. and Weintraub, H. (1975) Annu. Rev. Biochem. 44, 725–774.
[9] Kleinsmith, L. J., Stein, J. and Stein, G. (1976) Proc. Natl. Acad. Sci. USA 73, 1174–1178.
[10] Kuhen, G. D., Affoller, H. U., Atmar, V. J., Seebeck, T., Gubler, U. and Braun, R. (1979) Proc. Natl. Acad. Sci. USA 76, 2541–2545.
[11] Amalric, F., Zalta, J. P. and Simard, R. (1969) Exp. Cell. Res. 55, 370–376.
[12] Bouche, G., Amalric, F., Caizergues-Ferrer, M. and Zalta, J. P. (1979) Nucleic Acids Res. 7, 1739–1747.
[13] Zalta, J. and Zalta, J. P. (1973) Methods Cell Biol. 6, 317–324.
[14] Roeder, R. G. and Rutter, W. J. (1970) Proc. Natl. Acad. Sci. USA 65, 675–690.
[15] Amalric, F., Nicoloso, M. and Zalta, J. P. (1972) FEBS Lett. 22, 67–72.
[16] Stanley, P. E. and Williams, S. G. (1969) Anal. Biochem. 29, 381–392.
[17] Caizergues-Ferrer, M., Bouche, G., Amalric, F. and Zalta, J. P. (1980) Eur. J. Biochem. in press.