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EFFECT OF HEAT SHOCK ON RNA POLYMERASE ACTIVITIES IN CHINESE HAMSTER OVARY CELLS.

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SUMMARY

Incubation of Chinese hamster ovary cells at supranormal temperature (1 h at 43°C) results in an inhibition of high molecular weight RNA (rRNA and hnRNA) synthesis while the low molecular weight RNAs transcribed by RNA polymerase C (III) are still synthesized. In "in vitro" assays, the three RNA polymerase activities are detectable in extracts of heat shocked cells but are present at lower specific activities than in control cells (A (I):50 %; B (II): 81 %; C (III) : 76 %). Correlations have been established respectively between the "in vivo" RNA polymerase A and B activities and the level of phosphorylation of two proteins, one specific of the nucleolus (95,000 Mr) and one specific of the non nucleolar fraction of the nucleus (54,000 Mr). In their phosphorylated form, these two proteins could be specific "in vivo" inhibitors of the initiation of transcription by RNA polymerase I and II.

INTRODUCTION

An important advance in the knowledge of gene expression in eukaryotic cells has been the discovery of three classes of DNA dependent RNA polymerases which are specialized in the synthesis of defined RNAs (1, 2). However, the mechanism of regulation remains for the most part unknown, due to the failure of cell free system to carry out specific "in vitro" transcription. Recently, Wu (3) and Weil et al. (4) have shown that specific and different fragments of human adenovirus 2 DNA are selectively transcribed by RNA polymerase C (III) or RNA polymerase B(II). More recently, Wasylyk et al.(5) have obtained specific initiation of transcription of conalbumin and ovalbumin genes in a cell free system containing

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RNA polymerase B. A similar result has been reported by Grummt (6) using a cloned ribosomal DNA fragment containing the site of initiation of transcription, in the presence of RNA polymerase A. In each case, the "in vitro" transcription systems require cell free extracts of undefined composition which contain factors necessary for specific initiation of transcription. A partial characterization of these components has been carried out in systems in which specific genes are transcribed by RNA polymerase C (7,8). Following results from "in vivo" experiments, many hypotheses have been advanced to explain the regulation of transcription. These involve amino-acid concentration (9, 10), nucleotide pool size (11), stimulatory proteins (1) and protein phosphorylation (12, 13).

We have developed a heat shock system using CHO cells (14, 15) which appears to be particulary suitable for investigating the regulation of transcription "in vivo". RNA polymerase A and B can be successively turned on and turned off while RNA polymerase C is not affected. In non specific "in vitro" assays, the three RNA polymerases extracted from heat shocked and control cells present similar activities. In this experimental system, a partial characterization of factors that change according to the level of transcription has been carried out (14 - 16). Two proteins which vary in their level of phosphorylation could be specific factors involved in the regulation of the initiation of transcription by RNA polymerases A and B.

MATERIALS AND METHODS

The culture of CHO cells in monolayers has been previously described (16). Heat shock experiments, cell labelling and cell fractionation were carried out according to Caizergues-Ferrer et al. (16). The procedure for RNA extraction from purified nuclei or nucleoli was derived from that of Caboche and Bachellerie (17). Gel electrophoresis of the RNA samples was performed according to Tiollais et al. (18) for the analysis of high molecular weight species, and according to Maniatis et al. (19) for the analysis of low molecular weight RNA. RNA polymerases were extracted from purified nuclei by high salt (20). The assays were carried out as previously described (21) using exogenous calf thymus DNA as a template. Proteins were extracted from purified nucleoli, or from the non nucleolar part of nuclei and analyzed according to Bouche et al. (14).

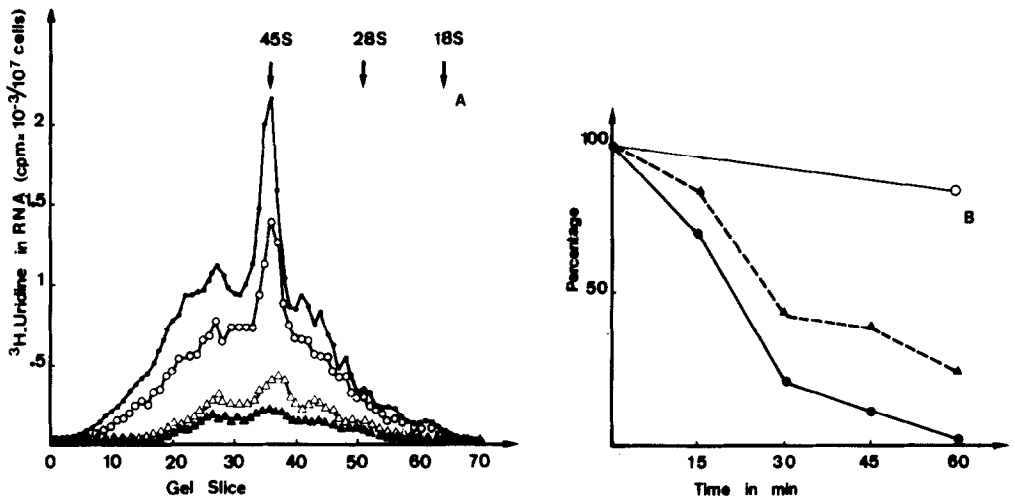

FIGURE 1 : Effect of heat shock on high molecular weight RNA synthesis

Cells were labeled for 15 min. with ^{3}H uridine (1 µCi/ml, 50 Ci/mole) at 37°C (●—●) or at 43°C between 0-15 min (○—○), 15-30 min (△—△) and 30-45 min (▲—▲) of treatment. RNA was extracted from purified nuclei and analyzed by slab gel electrophoresis using agarose-acrylamide composite gels (0.5 % agarose, 1.7 % acrylamide) (A). Fig. 1B : Relative inhibition of rRNA (●—●) hnRNA (▲—▲) and low molecular weight RNA (○—○) synthesis as a function of incubation time at 43°C. rRNA synthesis : cpm recovered from the 45S species; hnRNA synthesis: cpm recovered from the heterogeneous species; low molecular weight RNA synthesis : determined in experiments shown in fig. 2: cpm in 4S + 4.5S + 5S RNAs.

RESULTS

RNA synthesis in cells incubated for 1 h at 43°C

Incubation of CHO cells for 1 h at 43°C results in a complete but reversible inhibition of high molecular weight RNA synthesis (15). As shown in figure 1A, the incorporation of uridine into high molecular weight nuclear RNA (hnRNA and 45S preribosomal RNA) decreases rapidly at 43°C. Labelled precursor added after 30 min. incubation at supranormal temperature penetrates normally into the cells and is only slightly incorporated into rRNA precursors and hnRNA (respectively 10% and 30% of control). On the other hand, the total synthesis of low molecular weight RNA is only slightly decreased (fig. 1B).

In additional experiments, we have analyzed the RNAs labelled for one hour at 37°C or at 43°C by slab gel electrophoresis which resolves

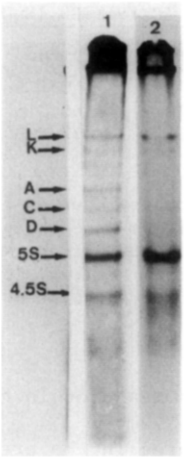

FIGURE 2 : Effect of heat shock on low molecular weight RNA synthesis

Cells were labeled for one hour with ^{3}H uridine at 37°C (1) or at 43°C (2). RNA was extracted from purified nuclei and analyzed by slab gel electrophoresis (10 % acrylamide). After the electrophoresis, the gels were treated for fluorography with Enhance (New England Nuclear) dried and then placed at -80°C with a X-OMAT R film (Kodak).

various low molecular weight RNAs synthesized by RNA polymerase A or B ((A, C, D species (22) : nomenclature of Penman (23)) and by RNA polymerase C (L, K, 5S,4S species (22)). As shown in figure 2, all the RNAs transcribed by RNA polymerase C are still synthesized at 43°C while the synthesis of species transcribed by RNA polymerase A or B is completely turned off. These results clearly show that RNA polymerases A and B are no longer active in cells incubated at 43°C (whatever the RNA species to be transcribed) while RNA polymerase C is not affected.

In vitro assays of RNA polymerases of heat shocked cells

According to "in vivo" experiments, RNA polymerases A and B are inactivated by the heat shock. This could result from a thermosensitivity of the enzymes or of a cofactor necessary for the initiation of transcription. To check between these two hypothesis RNA polymerase activities were measured "in vitro" in extracts from heat shocked and control cells. Nuclei were prepared and soluble enzyme activity was detected in the nuclear su-

TABLE 1 : RNA POLYMERASE ACTIVITIES IN CELLS INCUBATED AT 43°C.

Polymerase	Soluble	Bound	Total
A (I)	—	50 ± 5	50 ± 5
B (II)	125 ± 10	60 ± 5	81 ± 12
C (III)	95 ± 6	72 ± 6	76 ± 10

Cells were incubated for one hour at 43°C. Nuclei were prepared and "soluble" RNA polymerase activities were assayed on nuclear supernatant. For the determination of the "bound" activities, RNA polymerases were extracted from nuclei by sonication in high salt(20). Results are expressed as percentages of activity determined on the same number of control cells.

pernatant . The bound enzyme was measured in crude nuclear extract. RNA polymerase activities were measured in the presence of 200 µg/ml, 0,1 µg/ml or no ∝-amanitin to obtain a rough determination of the relative ratio of the three classes of RNA polymerase activities. As shown in Table 1, RNA polymerases B and C are present in similar amount in heat shocked and in control cells. More B enzyme was present in the soluble form after heat shock. On the other hand, only 50 % of the A enzyme was recovered in treated cells. Thus, the observed "in vivo" inhibition of high molecular weight RNA synthesis is not merely a result of the inactivation of RNA polymerases A and B.

Effects of heat shock on chromatin proteins

As previously reported (14), incubation of CHO cells at 43°C induces discrete variations in nuclear protein content. High molecular weight species appear that may be involved in chromatin condensation (results not shown) as well as changes in phosphoprotein content. Newly phosphorylated proteins are released from nucleus or nucleolus by a brief digestion with micrococcal nuclease thus copurifying with transcriptional complexes containing RNA polymerases, DNA and nascent RNA. Only a few non histone proteinsare recovered in these fractions and their general pattern by electrophoretic analysis is similar in control and heat shocked cells. As shown in Fig. 3, three proteins, one specific of the nucleolus (95,000 Mr) and two specific of the non nucleolar fraction of

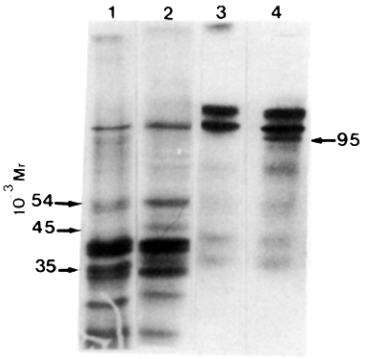

FIGURE 3 : Detection of proteins phosphorylated at 43°C

CHO cells were ^{32}P labeled for 1 h at 37°C or at 43°C. Nuclei were briefly digested with micrococcal nuclease (1 % TCA soluble) and centrifuged. A part of the proteins recovered in the supernatant were associated with transcriptional complexes (manuscript in preparation). A similar experiment was carried out with isolated nucleoli. Proteins from the two supernatants were TCA precipitated and analysed by electrophoresis on 10-16 % gradient slab gels (14). Gels were dried and exposed for 48 h for autoradiography. Lanes 1, 3 : control cells ^{32}P labeled for 1 h at 37°C. Lanes 2, 4 : cells ^{32}P labeled for 1 h at 43°C. Lanes 1, 2 : nuclear fraction; lanes 3, 4 : nucleolar fraction.

the nucleus (54,000 Mr and 45,000 Mr) become phosphorylated at 43°C. They remain phosphorylated during the recovery period at 37°C as long as high molecular weight RNA is not synthesized, then after 7 hours recovery, they are rapidly dephosphorylated as the synthesis of RNA is resumed (16). The treatment also induces the dephosphorylation of a 35,000 Mr protein that was shown to be associated with hnRNP and other minor variations in the phosphorylation level of several species.

DISCUSSION

The results obtained clearly show that inhibition of RNA synthesis by supranormal temperature does not simply result from an inactivation of RNA polymerase A and B. The apparent discrepancy observed between "in vivo" and "in vitro" results can have different interpretations :

1 - The modifications observed in chromatin structure (14) may induce a block of RNA chain elongation and possibly a release of RNA polymerases.

2 - RNA polymerases A and B need a thermosensitive factor for initiation or elongation or are specifically inhibited "in vivo" by a newly synthesized factor, possibly the phosphorylated proteins.

The observations reported here suggest that the first hypothesis is unlikely. The synthesis of RNAs with the same length, such as 5S RNA, and the L and K species on one hand, and the A, C and D species on the other hand, present different sensitivities to heat shock that can be directly related to the RNA polymerase species involved in the transcription process. However, it cannot be ruled out that phosphorylation of specific protein(s) result in discrete structural changes of chromatin domains around genes transcribed by RNA polymerase A and B. An alternative hypothesis is that the three proteins are cofactors of RNA polymerases. This is suggested by the strong correlation between RNA synthesis and the level of phosphorylation of the two proteins (95,000 Mr and 54,000 Mr) specific, respectively, of the nucleolar and non nucleolar fractions of the nucleus suggests that these two proteins are cofactors of RNA polymerases and that they are inactive in the phosphorylated form. Since they do not appear to be necessary for unspecific polymerase activities "in vitro", they could be involved in specific initiation or repression of transcription at promoter sites and not in elongation of RNA chains. According to published data (1) these two proteins are not components of the core enzymes. Since RNA polymerase C activity is not affected by the heat shock, this enzyme may not need such a cofactor. This interpretation would explain the "in vitro" transcription experiments in which it was demonstrated that specific initiation can be obtained using RNA polymerase C (4) while specific initiation with RNA polymerase A and B needed additional undefined proteins (3 - 6).

Purification of 95,000 Mr and 54,000 Mr proteins in phosphorylated and unphosphorylated forms is being carried out to study their role in specific "in vitro" transcriptional systems.

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REFERENCES

1. Chambon, P. (1975) Ann. Rev. Biochem. 44, 613-638
2. Roeder, R.G. in RNA polymerase (eds Losick, R. and Chambeslun, H.) 285-329 (Cold Spring Harbor Laboratory, New York, 1976).
3. Wu, G.J. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 2175-2179
4. Weil, P.A., Luse, D.S., Segall, J. and Roeder, R.G. (1979) Cell, 18 , 469-484
5. Wasylyk, B., Kedinger, C., Corden, J., Brison, O. and Chambon, P. (1980) Nature, 285, 367-373
6. Grummt, I., Personnal communication
7. Borkenmeier, E.H., Brown, D.D. and Jordan, E. (1978) Cell, 15, 1077-1086
8. Ng, S.Y., Parker, C.S. and Roeder, R.G. (1979) Proc. Nat. Acad. Sci. U.S.A., 76, 136-140
9. Holley, R.W. and Kierman, J.A. (1974) Proc. Nat. Acad. Sci. USA. 71, 2942-2945
10. Chesterton, C.J., Coupar, B.E.H., Butterworth, P.M.W., Buss, J. and Green, M.H. (1975), Eur. J. Biochem., 57, 79-84
11. Grummt, I. and Grummt, G., (1976) Cell, 7, 447-453
12. Kleinsmith, L.J. (1975) J. Cell. Physiol. 85, 459-475
13. Kuchn, G.D., Affoller, H.U., Atmar, V.J., Seebeck, J., Gubler, U. and Braun, R. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 2541-2545
14. Bouche, G., Amalric, F., Caizergues-Ferrer, M. and Zalta, J.P.(1979) Nucleic Acid Res. 7, 1739-1747
15. Caizergues-Ferrer,M.,Bouche, G., Amalric,F.(1980) Febs Letters, 116, 261-264
16. Caizergues-Ferrer, M., Bouche, G., Amalric, F. and Zalta, J.P. (1980) Eur. J. Biochem. 108, 399-404
17. Caboche, M. and Bachellerie, J.P. (1977) Eur. J. Biochem. 74, 19-29
18. Tiollais, P., Galibert, F., Lepetit, A. and Auger, M.A. (1972) Biochimie (Paris) 54, 339-354
19. Maniatis, T., Jeffrey, A. and Van de Sande, H. (1975) Biochemistry, 3787-3794
20. Roeder, R.G. and Rutter, W.J. (1970) Proc. Natl. Acad. Sci. U.S.A. 65, 675-690
21. Amalric, F., Nicoloso, M. and Zalta, J.P. (1972) Febs Letters, 22 , 67-72
22. Zieve, G., Benecke, B.J. and Penman,S. (1977) Biochemistry, 16, 4520-4525
23. Zieve, G. and Penman, S. (1976) Cell, 8, 19-31.