High salt effect on RNA synthesis in Krebs ascites tumor cells[1]

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Summary. The incubation of Krebs ascites tumor cells in medium with a high salt concentration resulted in a partial inhibition of nuclear RNA synthesis. The residual RNA polymerase activity in such nuclei was only slightly inhibited by low concentrations (50 nM) of α-amanitin. This finding suggested an inhibition of RNA polymerase II activity under conditions of medium hypertonicity. Indeed, RNA polymerase II, isolated from the nuclei of cells exposed to hypertonicity, revealed only half of the control activity. On the other hand, RNA polymerase I was not affected by hypertonicity. Moreover, chromatin fractions isolated from cells incubated in hypertonic or isotonic medium were equally template-active in RNA synthesis.

The control of eukaryotic gene expression has proved to be different from that of prokaryotic cells. Whereas the latter takes place mainly at the gene level during transcription, post-transcriptional control is also involved in the eukaryotic cell. The best-known examples of such regulatory mechanisms are the activation of a repressor of protein synthesis in reticulocyte lysates devoid of hemin, and that of similar translational inhibitors under the effect of interferon, double-stranded RNA or oxidized glutathione (for recent reviews, see Revel and Groner[3] and Safer and Anderson[4]). Though investigated less extensively, there are other cases in which the regulation also seems to take place also at the translational level, i.e., owing to changes in the incubation medium of cells, like renewal of the medium[5], elevation of the salt concentration[6-10], or of the incubation temperature[11-13]. These changes generally result in an inhibition of the initiation phase of protein synthesis. Among these, the inhibition caused by hypertonicity of the cell medium appears to show a relevance to the events in a cell during mitosis[6,7,14]. After exposure of the cells to hypertonicity a breakdown of the polysomes caused by an inhibition in initiation takes place[8-10] with the subsequent apparent masking of the released mRNA[7]. The results to be reported below suggest that, besides these changes at the translational level, hypertonicity causes an inhibition of RNA polymerase II.

Materials and methods. Incubation of cells. Krebs ascites tumor cells, kindly provided by Dr I. Horak, Würzburg, were grown in BALB/c mice. The harvested cells were collected by centrifugation for 2 min at 120×g and, thereafter, resuspended in minimal essential medium (Eagle) supplemented with 7% fetal calf serum. Alternatively, the cells were kept in the ascites fluid. (In both cases, the results obtained are in good agreement.) For experiments on hypertonicity, in either case, 4–5 ml of cell suspension containing ~ 10^7 cells/ml were divided into 2 halves; one of these was kept as the isotonic control (I) and the other was made hypertonic (H) (100 mM additional NaCl) by addition of an appropriate volume of 2 M NaCl to the medium. After incubation for 15 min at 37 °C under continuous shaking, the cells were again collected by centrifugation as above.

Preparation of nuclei and chromatin. The crude nuclear fraction obtained[10], was resuspended with a Dounce homogenizer in the homogenization buffer containing 1 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 20 mM Tris-HCl, pH 7.4, 410 mM sucrose and 0.5% Triton X-100. The nuclei were collected by centrifugation for 10 min at 1000×g, resuspended in 10 mM Tris-HCl, pH 7.4, 6 mM $MgCl_2$, 1 mM EDTA, 5 mM 2-mercaptoethanol and 30% glycerol and kept in liquid nitrogen. In order to isolate the chromatin, nuclei were distrupted in 20 mM EDTA-pH 7.4, 80 mM NaCl and 0.1 mM phenylmethanesulfonylfluoride (PMSF). The extracted chromatin was collected by centrifugation at 10,000×g for 15 min and resuspended in 80 mM NaCl, 10 mM Tris-HCl, pH 7.4 and 0.1 mM PMSF and centrifuged as above. All steps were repeated twice and the chromatin thus obtained was resuspended in 10 mM Tris-HCl, pH 7.4 and 0.1 mM PMSF and kept in liquid nitrogen.

Solubilization and fractionation of RNA polymerases. RNA polymerases were extracted from nuclei by sonication, treated with protamine sulfate and fractionated by DEAE-cellulose chromatography as described[15,16].

RNA synthesis. Activity in RNA synthesis was assayed with minor modifications as described[15]. The standard reaction mixtures for RNA synthesis contained in 0.1 ml 0.4 mM each ATP, GTP, CTP and 0.04 mM [^{14}C] UTP (The Radiochemical Center, Amersham), sp.act. 53 Ci/mole), 40 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 100 mM KCl and 14 mM 2-mercaptoethanol. After 15 min at 25 °C 75-µl aliquots were plated on GF/A (Whatman) filters and Cl_3CCOOH-precipitable radioactivity was determined. The amounts of nuclei or solubilized RNA polymerase proteins were as indicated in the legends. If α-amanitin (generously given by Dr T. Wieland, Heidelberg) was present, its concentration was 50 nM.

General methods. Protein determination was carried out according to Lowry[17] and that of DNA according to Burton[18].

Table 1. The effect of α-amanitin on RNA synthesis activity in nuclei from cells incubated with or without cycloheximide in isotonic (I), hypertonic (H) or first in hypertonic and then isotonic (IH) medium

System	[^{14}C]-UMP incorporated (pmoles/100 µl)		
	I	H	IH
Complete	85.9	62.3	85.8
+ α-amanitin	53.6	52.1	56.7
+ cycloheximide	98.2	70.3	88.4
+ α-amanitin and cycloheximide	50.8	38.0	42.7

Reaction conditions were as indicated in 'methods and materials'. 100 µl reaction mixture contained 4.2 A_{260} units of nuclei and, if present, 50 nM α-amanitin. Cycloheximide concentration in culture medium was 100 µM.

Table 2. The template activity of the chromatin fractions, from cells incubated in isotonic (I) or hypertonic (H) medium, as assayed in the presence of calf thymus RNA polymerase

System	[^{14}C]-UMP incorporated (pmoles/100 µl)	
	(I) chromatin	(H) chromatin
Complete	26.6	25.0
− RNA polymerase	6.9	6.9
+ α-amanitin	18.4	18.0

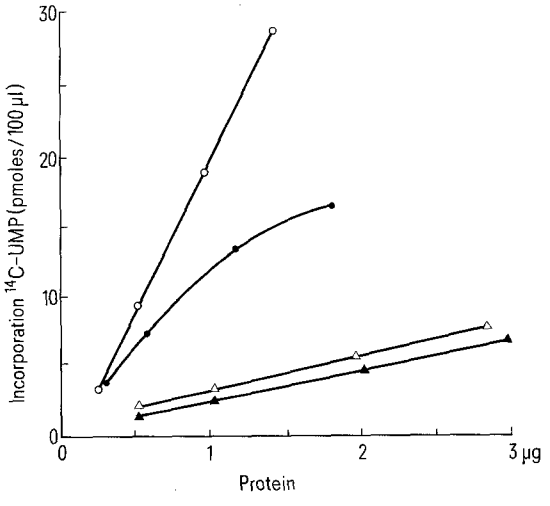
Assay conditions were as indicated in 'methods and materials'. 100 µl reaction mixtures contained 25 µg chromatin DNA and 32 µg calf thymus RNA polymerase protein (fraction III). If present, α-amanitin concentration was 50 nM.

Results. The nuclei from the cells incubated in hypertonic (H) medium were less active in RNA synthesis than those from control (I) cells. On the other hand, nuclei from cells passed from hypertonic to isotonic medium again (IH) displayed completely recovered activity, attesting to the reversibility of the inhibition caused by hypertonicity (table 1). Moreover, the residual activity in RNA synthesis in (H) nuclei was in comparison to the control activity only slightly depressed by low (50 nM) α-amanitin concentration. The presence of cycloheximide in the incubation medium increased the extent of RNA synthesis in all types of nuclei, probably because of the stimulation of the activity of RNA polymerase II[19,20]. However, the polymerase activity in the nuclei from the cells exposed to hypertonicity remained again under the control level. The addition of 50 nM α-amanitin depressed RNA synthesis and abolished the cycloheximide-promoted stimulation in all cases.

As shown in table 2, there was no difference between the template activities of the chromatin fractions from normal or hypertonic nuclei as assayed in the presence of heterologous calf thymus RNA polymerase fraction. This finding contrasted with some other cases where RNA synthesis seems to be regulated through changes in the chromatin structure which hinder the binding of RNA polymerase to the template[21].

Moreover, there was no difference observed in the activity of RNA polymerase I from cells incubated in either isotonic or hypertonic medium (fig.). However, the RNA polymerase II from the cells incubated in hypertonic medium displayed only half of the control activity.

Discussion. The effects of medium hypertonicity on gene expression appear to be complex according to the findings reported above. Besides the inhibition of protein synthesis[6-10], a partial inhibition of nuclear RNA polymerase activity occurs. Medium hypertonicity causes an inhibition of nuclear RNA polymerase activity which is sensitive to low (50 nM) α-amanitin concentrations. Moreover, RNA polymerase II fractionated from the nuclei of cells exposed to hypertonicity reveals only half of the control activity.



Dependence of RNA synthesis on the amounts of RNA polymerase I or II fractions added. Decrease in the activity of RNA polymerase II from cells exposed to hypertonicity. Experimental procedure was as described under 'methods and materials'. RNA polymerase I and II were separated by chromatography on DEAE-cellulose[16]. △—△, Isotonic RNA polymerase I; ▲—▲, hypertonic RNA polymerase I; ○—○, isotonic RNA polymerase II; ●—●, hypertonic RNA polymerase II.

This reduced activity does not appear to result from a leakage of RNA polymerase II from (H) nuclei since an activity sensitive to low (50 nM) α-amanitin concentrations was not found in the postnuclear supernatant fraction (not shown). Moreover, as shown in an early communication[22], a leakage due to nuclear damage is apt to involve RNA polymerase I rather than II. An additional inhibition of RNA polymerase III seems also unlikely on account of the sensitivity of inhibited activity to low α-amanitin concentrations. Thus, we assume that the nuclear component of RNA synthesis inhibited predominantly by the medium hypertonicity corresponds to RNA polymerase II.

The inhibition of RNA synthesis might be due to the primary inhibition of protein synthesis, i.e. of a component of RNA polymerase II turning over rapidly. However, the observed stimulation of α-amanitin-sensitive RNA polymerase activity after the addition of cycloheximide to the hypertonic cell medium implicates a different mechanism, provided cycloheximide exerts its effect solely at the translation level[23]. Also, cycloheximide, employed at concentrations not fully inhibitory for protein synthesis, has been shown to stimulate RNA synthesis in aminoacid starved cells[24].

The relationship of hypertonicity-mediated changes to the events occurring during cell division remains to be established. Besides some morphological similarities, i.e. chromatin condensation[6,7], both cases display a block in the initiation of protein synthesis concomitant with an inhibition of RNA synthesis[6-10,14,25,26]. Thus, work on hypertonicity-mediated changes in cells might provide further insight into the cellular control mechanisms during mitosis.

1 This investigation was supported by the Turkish Scientific and Technical Research Council (TÜBİTAK), Project No. TAG 339.
2 Biyoloji Bölümü, Temel Bilimler Fakültesi, Boğaziçi Üniversitesi, Istanbul.
3 M. Revel and Y. Groner, A. Rev. Biochem. *47*, 1079 (1978).
4 B. Safer and W.F. Anderson, CRC Crit. Rev. Biochem. *5*, 261 (1978).
5 J.M. Whelley and R. Baserga, Biochim. biophys. Acta *425*, 234 (1976).
6 C. Robbins, T. Pederson and P. Klein, J. Cell Biol. *44*, 400 (1970).
7 G. Wengler and G. Wengler, Eur. J. Biochem. *27*, 162 (1972).
8 J.L. Saborio, S.S. Pong and G. Koch, J. molec. Biol. *85*, 195 (1974).
9 D.L. Nuss, H. Offermann and G. Koch, Proc. natl Acad. Sci. USA *72*, 1258 (1975).
10 M.C. Robertson and E. Bermek, Biochim. biophys. Acta *476*, 88 (1977).
11 W. McCormick and S. Penman, J. molec. Biol. *39*, 315 (1969).
12 J. Schochetman and R.P. Perry, J. molec. Biol. *63*, 577 (1972).
13 S.A. Austin and J.E. Kay, Biochim. biophys. Acta *395*, 468 (1975).
14 H. Fan and S. Penman, J. molec. Biol. *50*, 655 (1970).
15 C. Kedinger, F. Gissinger, M. Gniazdowski, J.L. Mandel and P. Chambon, Eur. J. Biochem. *28*, 269 (1972).
16 S. Cereghini and F.T. Fernandez, FEBS Lett. *41*, 161 (1974).
17 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
18 K. Burton, Biochem. J. *62*, 315 (1956).
19 J.J. Chik, L.M. Pike and T.M. Devlin, Biochem. J. *168*, 501 (1977).
20 J.T. Lindell and J.J. Duffy, J. biol. Chem. *254*, 1454 (1978).
21 C.J. Chesterton, B.E.H. Coupar, P.H.W. Butterworth, J. Buss and H.M. Green, Eur. J. Biochem. *57*, 79 (1975).
22 K.H. Seifart, B.J. Benecke and P.P. Juhasz, Archs Biochem. Biophys. *151*, 519 (1972).
23 D. Vazquez, FEBS Lett. *40(S)*, 63 (1974).
24 A.O. Pogo and V.J. Zbrzezna, FEBS Lett. *88*, 135 (1978).
25 I.E. Feinendigen and B.P. Bond, Exp. Cell Res. *30*, 393 (1963).
26 H. Fan and S. Penman, J. molec. Biol. *59*, 27 (1971).