Vol. 123, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1984 Pages 78-83

# RNA-CONTAINING NUCLEAR BINDING SITES FOR GLUCOCORTICOID-RECEPTOR COMPLEXES

Gian Paolo Rossini

Istituto di Chimica Biologica, Università di Modena,
Via Campi 287, 41100 Modena (Italy)

Received June 4, 1984

RNase A treatment of HeLa cell nuclei causes a time- and concentration-dependent release of dexamethasone-receptor complexes. If nuclei are incubated in the absence of enzyme, only 60% of RNase-releasable complexes can be detected. Sucrose density gradient analysis of nuclear extracts shows that receptor complexes released by RNase treatment sediment at 3.6 S, whereas complexes obtained from untreated nuclei sediment between 7 and 3.6 S. Our results show that a fraction of dexamethasone-receptor complexes retained by HeLa cell nuclei is located in binding sites involving RNA.

In the currently accepted model of steroid hormone action, the interaction of steroid-receptor complexes with chromatin is believed to regulate the synthesis of certain RNAs (1). It has not been established, however, whether steroid-receptor complexes might also exert an action at a post-transcriptional level. In 1969, Liao proposed that steroid-receptor complexes might be released from chromatin in association with RNA (2). This contention has been later supported by the findings that androgen- and estrogen-receptor complexes can interact with nuclear ribonucleoprotein particles (3,4), and that RNA can cause the release of steroid-receptor complexes from DNA *in vitro* (5,6). By mild sonication of HeLa cell nuclei, we have reported evidence on the possible existence of nuclear glucocorticoid-receptor-RNA complexes (7). That interaction, however, might have been caused by the procedure employed in the preparation of nuclear extracts, and the existence of RNA-associated glucocorticoid-receptor complexes in HeLa cell nuclei remained questionable. We here report that part of glucocorticoid-receptor complexes retained by HeLa cell nuclei is located in binding sites involving RNA.

0006-291X/84 $1.50

## EXPERIMENTAL PROCEDURES

Materials. [$^3$H]-Dexamethasone (46 Ci/mmol) was purchased from New England Nuclear. Ovalbumin (OV, 3.5 S), porcine IgG (6.6 S), unlabeled dexamethasone, and pancreatic RNase A were obtained from Sigma.

Cell culture and isotope labeling. HeLa $S_3$ cells were grown in suspension culture (7). To obtain chromatin-bound glucocorticoid-receptor complexes, HeLa cells were incubated for 15 min at 37°C with 50 nM radioactive dexamethasone, and in the presence, or absence, of 5 μM unlabeled dexamethasone.

Cell fractionation. Cells pulsed with radioactive dexamethasone were washed, and homogenized to obtain crude nuclei, as previously described (7). The nuclear preparation was then washed three times with 10 mM Tris/HCl, pH 7.5, 10 mM NaCl, 1.5 mM $MgCl_2$ (TNM), and resuspended in the same medium before extraction of dexamethasone-receptor complexes. The salt extraction of nuclear complexes was carried out by addition of 1.5 M NaCl in TNM buffer up to a final 0.3 M NaCl concentration. The suspension was then incubated for 1 hr at 2°C before a soluble extract was obtained by centrifugation for 15 min at 15,000 x g. In most experiments dexamethasone-receptor complexes were obtained by incubation of washed nuclei in 3-10 volumes of TNM buffer at 2°C, and in the presence, or absence, of RNase A, as described in the text. A soluble extract was then obtained by centrifuging the nuclear suspension for 15 min at 15,000 x g.

Other procedures. Dexamethasone-receptor complexes in soluble extracts from HeLa cell nuclei were analyzed and quantified by sucrose density gradient centrifugations, as previously described (7). Radioactivity was measured in a Packard liquid scintillation spectrometer, with a toluene-based liquid scintillation fluid, as previously described (5). The counting efficiency was about 35%. DNA was measured by the diphenylamine test, with calf thymus DNA as the standard (8). Results shown in figures have been taken from one of the three or more replicate experiments performed.

## RESULTS AND DISCUSSION

To investigate on the possible existence of RNA-associated glucocorticoid-receptor complexes, HeLa cell nuclei were preliminarily incubated in the presence of increasing amounts of RNase A, and the soluble extracts thus obtained were subjected to high salt sucrose density gradient centrifugations. The results presented in Figure 1A show that RNase treatment led to a concentration-dependent release of dexamethasone-receptor complexes in the incubation medium. In the absence of the enzyme, however, receptor complexes were still detected in the medium, and these complexes accounted for about 60% of the amounts detectable after RNase A treatment of paired nuclear suspensions.

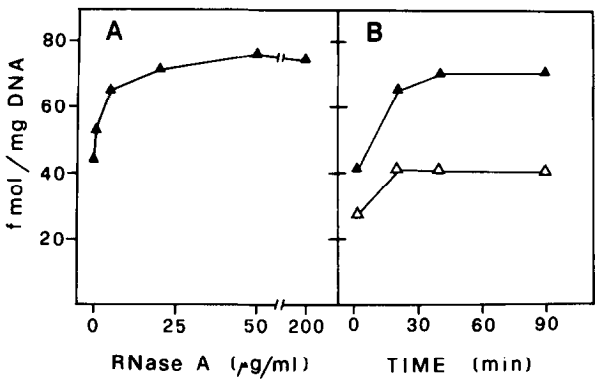


Fig. 1. (A) Effect of increasing RNase A concentrations on the detection of dexamethasone-receptor complexes in the soluble extracts obtained from HeLa cell nuclei. Nuclear suspensions were incubated for 40 min at 2°C in the presence of the indicated amounts of enzyme. (B) Time-course of dexamethasone-receptor complex release in soluble extracts from HeLa cell nuclei. Nuclear suspensions were incubated at 2°C for the indicated times, and in the presence (solid triangles), or absence (open triangles) of RNase A (200 μg/ml).
Analysis and quantification of dexamethasone-receptor complexes in soluble extracts from nuclear suspensions were performed by high salt sucrose density gradient centrifugations.

The time-course of the nuclear release of dexamethasone-receptor complexes is shown in Figure 1B. During the incubation of HeLa cell nuclei with buffer, receptor complexes appeared to be released in the medium within 30 min. The presence of RNase A during the incubation did not significantly affect the overall trend of the phenomenon. This treatment, however, caused a 75% increase in the released dexamethasone-receptor complexes (Fig. 1B). RNase S-protein, a catalytically inactive fragment of RNase A (9), could not cause the release of 3.6 S receptor complexes that we observed with the active enzyme.

The leakage of nuclear complexes into the buffer did not appear to be simply due to the partition of receptor complexes between nuclear and extranuclear compartments. In fact, by increasing the dilution of resuspended nuclei we could not cause a parallel increase, as judged on a DNA basis, in the amounts of complexes released during the incubation.

Maximal amounts of dexamethasone-receptor complexes extractable by RNase treatment were 78.8 $\pm$ 6.7 fmol/mg DNA of nuclear suspensions (mean $\pm$ SEM, n=5), representing about 10% of total nuclear complexes extractable by 0.3 M NaCl. The remaining 90% could be obtained by salt extraction of RNase-treated nuclei.

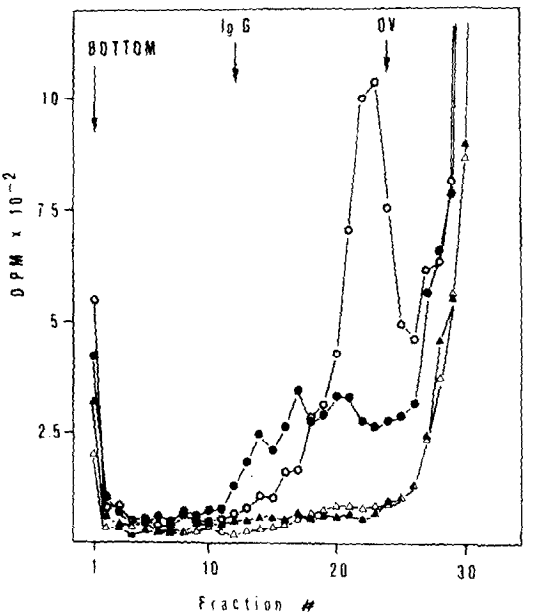


Fig. 2. Sucrose density gradient centrifugation of soluble extracts from HeLa cell nuclei. HeLa cells were incubated for 15 min at 37°C with 50 nM radioactive dexamethasone, and in the presence (triangles), or absence (circles) of 5 μM unlabeled dexamethasone. Washed nuclei were resuspended with TNM buffer and were incubated for 40 min at 2°C in the presence (open symbols), or absence (closed symbols) of RNase A (200 μg/ml). Portions of the soluble extracts, corresponding to the same amounts of DNA, were then analyzed by low salt sucrose gradients.

To further study dexamethasone-receptor complexes, soluble extracts from HeLa cell nuclei were analyzed by low salt sucrose density gradient centrifugations. As shown in Figure 2, the extracts obtained from RNase-treated nuclei contained dexamethasone-receptor complexes which homogeneously sedimented at 3.6 S. Under low salt conditions, however, receptor complexes in the extracts from untreated (control) nuclei heterogeneously sedimented between 7 and 3.6 S (Fig. 2). These results, therefore, indicated that soluble extracts from control nuclei contained dexamethasone-receptor complexes associated to some component(s). As judged by sucrose density gradient centrifugation, however, RNase A treatment of HeLa cell nuclei abolished the appearance of heterogeneously sedimenting receptor complexes. These findings suggested that dexamethasone-receptor-RNA complexes could be present in soluble extracts from control nuclei. To test this hypothesis, soluble extracts from control nuclei were treated with hydrolytic enzymes before being analyzed by low salt sucrose density gra-

dient centrifugation. As we have shown in the case of soluble extracts from sonicates of HeLa cell nuclei (7), heterogeneously sedimenting receptor complexes could be completely converted to the 3.6 S form by RNase A treatment, whereas this was not the case when pancreatic DNase, or pepsin, substituted for RNase A (not shown). Moreover, RNase S-protein was unable to convert receptor complexes to the 3.6 S form. We concluded, therefore, that some RNA(s) must be associated with dexamethasone-receptor complexes in soluble extracts from control nuclei, whose destruction led to recovery of 3.6 S receptor complexes.

The time- and concentration-dependent, RNase A-induced release of dexamethasone-receptor complexes from HeLa cell nuclei indicates that part of nuclear complexes are located in binding sites involving RNA. Moreover, the RNase A-dependent conversion of heterogeneously sedimenting receptor complexes in soluble extracts from untreated nuclei indicates that the population of receptor complexes in HeLa cell nuclei includes RNA-associated dexamethasone-receptor complexes. Thus, these entities do not appear to result from methodological artifacts (7). It should be noted that these complexes appear to be distinct from cytosolic RNA-associated receptor complexes (G. P. Rossini, in preparation) inasmuch as the sedimentation properties of the two receptor complex populations do not coincide in both RNase A-treated, and untreated samples.

The nuclear leakage of RNA-associated receptor complexes suggests that a precise estimate of their nuclear levels might not have been achieved. Under our experimental conditions, RNase A-releasable complexes accounted for about 1,000 binding sites per cell. An accurate estimate of nuclear RNA-associated steroid-receptor levels would be highly relevant to the possible physiological role of this interaction. The existence of nuclear steroid-receptor-RNA complexes is implied in Liao's hypothesis on the physiological role of RNA-receptor complex interactions (2). In that model, RNA binding to steroid-receptor complexes would cause the release of chromatin-bound receptor complexes. Our findings are in agreement with this hypothesis. Although nuclear levels of RNA-associated dexamethasone-receptor complexes in HeLa cells might be larger than our estimates, the amounts we detected cannot be a marginal fraction of nuclear complexes. If, as it has been proposed, recep-

tor complexes are released from DNA in association with RNA (2), and if RNA-steroid-receptor complexes are recycled (3, 10), it seems likely that, at any instant, only a fraction of nuclear complexes can be found associated to RNA. Moreover, the nuclear leakage of RNA-associated dexamethasone-receptor complexes would indicate that part of these entities are either soluble, or loosely associated to chromatin.

The identification of an RNA-associated population of dexamethasone-receptor complexes in HeLa cell nuclei will allow further studies to evaluate Liao's hypothesis on the functional role of RNA-steroid-receptor complexes in target cell nuclei.

Acknowledgments. This work was supported by a grant from the italian Ministero della Pubblica Istruzione.

REFERENCES

1. Gorski, J., and Gannon, F. (1976) Ann. Rev. Physiol. 38, 425-450.
2. Liao, S., and Fang, S. (1969) Vit. Horm. 27, 17-90.
3. Liao, S., Liang, T., and Tymoczko, J. L. (1973) Nature New Biol. 241, 211-213.
4. Liang, T., and Liao, S. (1974) J. Biol. Chem. 249, 4671-4678.
5. Liao, S., Smythe, S., Tymoczko, J. L., Rossini, G. P., Chen, C., and Hiipakka, R. A. (1980) J. Biol. Chem. 255, 5545-5551.
6. Tymokzko, J. L., Shapiro, J., Simenstad, D. J., and Nish, A. D. (1982) J. Steroid Biochem. 16, 595-598.
7. Rossini, G. P., and Barbiroli, B. (1983) Biochem. Biophys. Res. Commun. 113, 876-882.
8. Burton, K. (1956) Biochem. J. 62, 315-323.
9. Richards, F. M., and Wyckoff, H. W. (1971) The Enzymes (Boyer, P. D., Ed.), Vol. 4, pp. 647-806, Academic Press, New York.
10. Rossini, G. P. (1984) J. Theor. Biol. 108, in the press.