HETEROGENEITY OF CHROMOSOMAL RNA

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1. Introduction

It has been found in several tissues that part of the RNA of chromatin is associated with chromosomal proteins. This RNA, usually designated chromosomal RNA, is claimed by Bonner and his associates [1, 2] to constitute a specific, well defined class of RNA which is covalently bound to proteins and is assumed to play an important role in gene regulation. Recently, however, doubts have been raised with regard to the homogeneity and significance of chromosomal RNA. Thus, Heyden and Zachau [3] reported that chromosomal RNA, isolated from calf thymus, consisted to a large extent of degraded tRNA, and Artman and Roth [4] found no evidence for the existence in chicken tissues of a specific low-molecular RNA with the properties described for chromosomal RNA. Moreover, we have recently obtained evidence [5] that only a very small portion of the DNA of rat liver may be present in the form of DNA–RNA hybrids, a finding which does not support the hypothesis [1, 6, 7] that chromosomal RNA functions as a sequence detector for chromosomal proteins.

In the present paper data are presented, indicating that the RNA moiety of chromosomal RNA from rat liver is not a well defined entity, but consists of different types of RNA, the major part of which is not chemically bound to the protein.

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2. Materials and methods

Female Wistar rats weighing 150–170 g were used. The animals were injected with 1 mCi of ^{3}H-orotic acid (Radiochemical Centre, Amersham, England), starved for 24 hr and killed by decapitation. The livers were excised, the nuclei were isolated according to Blobel and Potter [8] and the chromatin was prepared as described by Huang and Huang [7]. The preparations were stored in liquid nitrogen until use.

2.1. *Fractionation of chromatin*

Chromatin was fractionated by dissociation in 4 M CsCl as described by Bonner and Widholm [9]. The skin, supernatant, and pellet fractions obtained after centrifugation were washed free of CsCl by 75% ethanol containing 0.2 M NaCl. The fractions were homogenized in a motor-driven Potter-Elvehjem homogenizer in 0.01 M Tris buffer pH 8 or under the conditions described in fig. 1.

2.2. *Analytical methods*

RNA was determined by the orcinol method [10] in alkaline hydrolysates of the different fractions [11]. DNA was determined by the diphenylamine reaction [12]. Histones and the non-histone proteins were separated according to Bonner et al. [13] and determined by the method of Lowry et al. [14].

To determine the radioactivity, the RNA was precipitated with trichloroacetic acid (final concentration 5%) and subsequently 500 μg of bovine serum albumin were added. The precipitates were collected on glass fiber filters and the samples were counted as previously described [15].

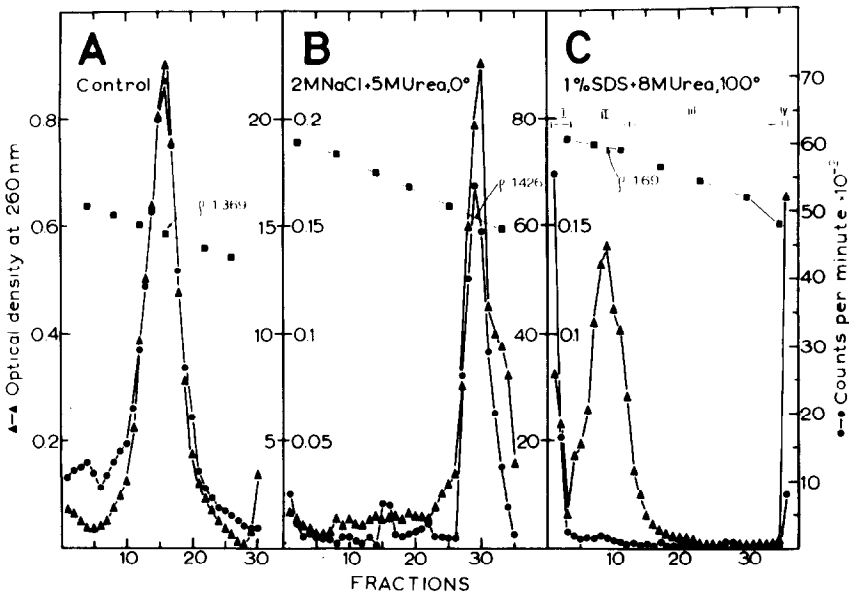

Fig. 1. Dissociation of the skin fraction of rat liver chromatin. The skin fraction was homogenized and treated as indicated below, and mixed with solid CsCl. Isopynic CsCl gradient centrifugation was carried out at 37,000 rpm for 60 hr in an SW 40 rotor in a Beckman L2 ultracentrifuge. The absorbance at 260 nm and the radioactivity were determined in aliquots of the fractions. A) Skin homogenized in 0.01 M Tris buffer, pH 8. Final concentration of CsCl 3.2 M. B) Skin homogenized in 2 M NaCl + 5 M urea in sodium acetate buffer, pH 6, and incubated at 0° for 15 min. Final concentration of CsCl 4.6 M. C) Skin fraction homogenized in 1% SDS + 8 M urea, and incubated for 15 min at 100°. Excess amounts of potassium chloride were added and the system was centrifuged at 10° at 15,000 rpm for 20 min to remove the SDS. Final concentration of CsCl 4.6 M.

3. Results and discussion

When crude chromatin is dissolved and centrifuged in 4 M CsCl, the protein-associated RNA, "chromosomal RNA", floats to the top to form a pellicle or skin fraction [9]. A representative experiment showing the distribution of the DNA and RNA in the fractions obtained from rat liver chromatin by this procedure is given in table 1. It is seen that the dissociation of the nucleoprotein was not complete as the main part of DNA was found in the supernatant fraction and not in the pellet. Moreover, the skin fraction contained more DNA than RNA. Most of the labelled RNA was found in the pellet.

Attempts were made to dissociate the RNA–protein complex of the skin fraction by different treatments and subsequent isopycnic centrifugation in CsCl. It is seen from fig. 1A that the untreated nucleoprotein complex banded at a buoyant density of 1.369. After treatment of the skin with 2 M NaCl and 5 M urea, the optical density, as well as the radioactivity, banded at $\rho = 1.428$. Thus, although some protein was liberated by the treatment, no free RNA or DNA was released. Treatment of the same material for 15 min at 0° with 1% sodium dodecyl sulphate (SDS) gave similar results (data not shown). The results indicate that the RNA and DNA of the skin fraction are rather firmly associated with the protein. When, however, the skin was dispersed in a mixture of 1% SDS and 8 M urea and incubated at 100° for 15 min, a drastic change in the distribution pattern was observed (fig. 1C). The optical density showed a large peak at $\rho = 1.69$, close to the buoyant density of pure DNA. Chemical analysis of the pooled fractions from this peak (fraction II) confirmed that it contained most of the DNA of the skin (data not shown). The major part of the radioactivity was found in the pellet. Since the pellet had a buoyant density in excess of $\rho = 1.75$, this indicates that RNA had been liberated by the treatment. On the surface

Table 1
Distribution of DNA and RNA in fractions from rat liver chromatin*.

Fraction	DNA	RNA		
	(μg)	(μg)	(cpm x 10^{-3})	Specific activity (cpm/μg)
Whole chromatin	1740 (100%)	257 (100%)	1180	4390
Skin fraction ("chromosomal RNA")	78 (4.5%)	64 (24.9%)	47.8	749
Supernatant	1140 (65.4%)	35.7 (13.9%)	34.5	364
Pellet	155 (8.9%)	124 (48.2%)	846	6830

* The chromatin was dissociated in 4 M CsCl as described in Materials and methods. The relative content of RNA histone and non-histone protein, setting DNA equal to 1, was: 0.069, 0.917 and 0.55, respectively.

Table 2
Distribution of labelled RNA in fractions obtained after CsCl centrifugation of the skin fraction of rat liver chromatin treated with SDS and urea.

Fractions	RNA		
	(μg)	(cpm x 10^{-3})	Specific activity (cpm/μg)
I	54.7	150	2 720
II	18.5	67.1	3 610
III	46.2	7.5	162
IV	25.5	27.1	1 060

The pooled fractions from the experiments shown in fig. 1C were precipitated with 2 vol of ethanol and left overnight at −20°. After removal of CsCl by several washings in 75% ethanol containing 0.2 M NaCl, the RNA content and the radioactivity were determined. The specific activity of the skin fraction was 1200 cpm per μg of RNA.

of the gradient a voluminous pellicle was formed ($\rho < 1.4$) consisting primarily of protein, but also containing some radioactivity.

In table 2 data are given on the pooled fractions (Roman numerals of fig. 1C). The results reveal that all fractions contained significant amounts of RNA, the specific activity of which differed widely. RNA of fractions I and II had a high specific activity, while that of fraction III was very low and that of fraction IV showed an intermediate value. Since fraction III sedimented at buoyant densities lower than 1.6 its RNA was associated with appreciable amounts of proteins. The very low specific activity of its RNA shows that it is distinctly different from that of the other fractions. When fraction IV was submitted to a second treatment with SDS and urea at 100° and recentrifuged in CsCl, the major part of the radioactivity pelleted, indicating that most of its RNA was liberated. It thus appears that although a definite amount of chromosomal RNA was resistant to treatment with SDS and urea at 100°, the major part of the labelled RNA of the skin could be released from the protein by this treatment.

The present findings that the RNA moiety of chromosomal RNA consists of fractions with different specific activities and that about two thirds of the RNA can be dissociated from the protein by a procedure not expected to break covalent bonds, do not confirm previous reports [1, 2, 16]. The results, together with those of Heyden and Zachau [3], obtained on calf thymus chromatin, and the report by Artman and Roth [4] that, depending on the conditions of isolation, the quantity of chromosomal RNA of chicken tissues varied from zero to almost 100% of the total chromatin-associated RNA, provide strong evidence that "chromosomal RNA" is not a distinct and well defined class of RNA.

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References

[1] J. Bonner, M.E. Dahmus, D. Fambrough, R.C. Huang, K. Marushige and D.Y.H. Tuan, Science 159 (1968) 47.
[2] M.E. Dahmus and J. Bonner, Federal Proc. 29 (1970) 1255.
[3] H.W. v. Heyden and H.J. Zachau, Biochim. Biophys. Acta 232 (1971) 651.
[4] M. Artman and J. Roth, J. Mol. Biol. 60 (1971) 291.
[5] F. Szeszák and A. Pihl, Biochim. Biophys. Acta 247 (1971) 363.
[6] I. Bekhor, G.M. Kung and I. Bonner, J. Mol. Biol. 39 (1969) 351.
[7] R.C. Huang and P.C. Huang, J. Mol. Biol. 39 (1969) 365.
[8] G. Blobel and V.R. Potter, Science 154 (1966) 1662.
[9] J. Bonner and J. Widholm, Proc. Natl. Acad. Sci. US. 57 (1967) 1379.
[10] W.C. Schneider, in: Methods in Enzymology, Vol. III, eds. S.P. Colowick and N.O. Kaplan (Academic Press, New York, 1957) p. 681.
[11] L.M.H. Shaw and R.C. Huang, Biochemistry 9 (1970) 4530.
[12] K. Burton, Biochem. J. 62 (1956) 315.
[13] J. Bonner, G.R. Chalkley, M. Dahmus, D. Fambrough, F. Fujimura, R.C. Huang, J. Huberman, R. Jensen, K. Marushige, H. Ohlenbusch, B. Olivera, and J. Widholm, in: Methods in Enzymology, Vol. XII, eds. L. Grossman and K. Moldave (Academic Press, New York, 1968) p. 20.
[14] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[15] H.C. Birnboim, Anal. Biochem. 37 (1970) 178.
[16] R.C. Huang, Federal Proc. 26 (1967) 603.