FRACTIONATION OF CHROMATIN BASED ON STRENGTH OF BINDING WITH THE MATRIX

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By washing and subsequent centrifugation of rat liver nuclei in medium of low ionic strength the chromatin can be separated into two fractions: Chromatin eluting into the supernatant and chromatin firmly bound with the matrix. The chromatin fraction eluting into the supernatant contains about 60-70% of the original DNA and about 15% of the original protein. It has a complete set of histones and only a small quantity of nonhistone proteins. The RNA-synthesizing ability of chromatin firmly bound with the matrix is 2-3 times greater than that in the original nuclei. Data on gradient centrifugation do not allow the increase in uptake of label to be ascribed entirely to the approximately equal lengthening of chains of synthesized RNA. The increase in ability to synthesize RNA is evidently due to the larger number of molecules of RNA-polymerase per unit of DNA in the chromatin fraction firmly bound with the matrix. Comparison of the results with data in the literature indicates that the chromatin fraction bound with the matrix is enriched with transcribable genes.

KEY WORDS: matrix; chromatin; fractionation.

The matrix plays an essential role in the structural organization of nuclear chromatin [5]. Since structure and function of DNP preparations are closely interconnected [1], it can be suggested that differences in the ability of chromatin fractions to be separated from the matrix will correspond to differences in their functional state.

The object of this investigation was to fractionate chromatin on the basis of the strength of its binding to the matrix and to study the ability of these fractions to act as template for RNA synthesis.

EXPERIMENTAL METHOD

Nuclei were isolated from rat livers at pH 7.4 as described previously [3]. The nuclear residue was suspended in 10 mM Tris-Hcl, pH 7.4, 0.2 mM $MgCl_2$ (TM solution), with DNA present in a concentration of 1-3 mg/ml, mixed for 5 min, and centrifuged for 20 min at 2500 rpm on the K-23 centrifuge (East Germany). The residue was suspended in 20 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.25 M sucrose, 0.3 mM β-mercaptoethanol. Sometimes the procedure of washing in TM was repeated several times. RNA synthesis in the nuclei was investigated at low (5 mM $MgCl_2$) and high [0.2 M $(NH_4)_2SO_4$, 1 mM $MnSO_4$] ionic strengths [3]. For gradient ultracentrifugation the reaction of RNA synthesis was stopped by the addition of Na dodecyl sulfate (DDS) to 0.1% DDS, 0.1 M NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4. Sedimentation coefficients were calculated by the method described previously [4]. Electrophoresis of nuclear proteins was carried out in 15% polyacrylamide gel [8]. The number of nuclei was counted in a Goryaev's chamber. DNA and protein were determined as described previously [3].

EXPERIMENTAL RESULTS

Washing and centrifugation of rat liver nuclei under the conditions described in the "Experimental Method" section, depending on the intensity of mixing before centrifugation, led to the removal of up to 60-70% of the original DNA and 10-20% of the original protein from the supernatant. Electrophoretic spectra of proteins of

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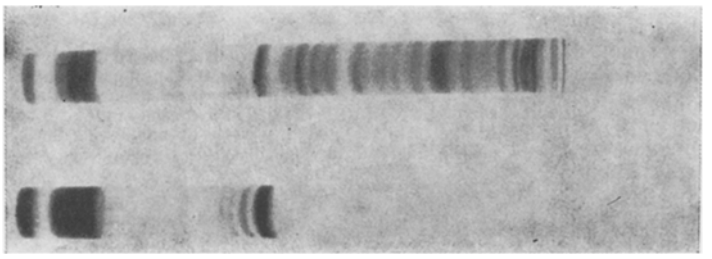

Fig. 1. Electrophoretic spectra of proteins. Left – proteins of original nuclei; right – proteins eluting into supernatant.

TABLE 1. Characteristics of Chromatin Fraction Firmly Bound with Matrix

Index studied	Number of washings					
	0	1	2	3	4	5
Number of residual nuclei, %	100	95	59	53	53	53
Residual DNA content, %	100	38	24	18	13	11
A_{230}/A_{260}	1,00	1,15	1,29	1,39	1,44	1,46
Incorporation of UMP-^{3}H [in the presence of Mn^{2+}, $(NH_4)_2SO_4$], %	100	160	330	330	330	330
Incorporation of UMP-^{3}H (in the presence of Mg^{2+}) %	100	200	200	200	160	180

the original nuclei and of those eluting into the supernatant are given in Fig. 1. They show that all histomes were present in the supernatant but virtually no nonhistone proteins. On the addition of up to 2mM $MgCl_2$ to this fraction, instantaneous aggregation was observed, evidence that DNA in this fraction was associated with histones. Subsequent washings enabled a further approximately 20% of DNA to be extracted from the nuclei, but its appearance was largely due to destruction of the nuclei. Under these circumstances nuclear nonhistone proteins appeared in the supernatant.

As Table 1 (the results of one typical experiment) show the RNA-synthesizing ability of the chromatin which remained firmly bound to the matrix after several washings was higher than that of the original nuclei. It is well known that virtually all RNA-polymerase in the nuclei is associated with chromatin, and that it can be separated from the complex only at alkaline pH values or in a medium with high ionic strength [6, 9]. The increase in the RNA-synthesizing ability was therefore due either to an increase in the number of RNA-polymerase molecules per unit of DNA or to a change in the superstructure of the chromatin as a result of removal of the greater part of the DNA. Since RNA synthesis is catalyzed by a limited number of enzyme molecules, evidently already associated with chromatin, changes in superstructure could lead either to an increase in the length of the RNA chains or to the appearance of additional initiation sites on the chromatin. However, under the conditions when virtually no reinitiation takes place (RNA synthesis in medium of low ionic strength) the RNA-synthesizing ability of the chromatin fraction firmly bound with the matrix was nevertheless much greater than that of the original nuclei.

To analyze the sedimentation coefficient of the synthesized RNA experiments with gradient ultracentrifugation were carried out. As a result of the relationship $M = 1550\ S^{2,1}_{20w}$, an on average 2-3-fold increase in molecular weight leads to an increase of 1.4-1.7 times in the sedimentation coefficient. However, in none of the experiments was the sedimentation coefficient increased by more than 1.2-1.3 times.

Consequently, the increase in RNA-synthesizing ability of the chromatin bound to the matrix was due mainly to the increased concentration of RNA-polymerase in this chromatin fraction and not to an increase in the chain length of the newly synthesized RNA.

What causes solubilization of such a high proportion of the DNA in chromatin? It may be that it is the action of endogenous nucleases [5], which are mainly associated with heterochromatin [10, 11]. However, the possibility cannot be ruled out that breaks facilitating its removal from the nuclei under these conditions are already present in DNA in vivo [12].

The chromatin fraction firmly bound to the matrix is thus enriched with nonhistone proteins. RNA-polymerase is also bound to this same fraction. It is well known that the active chromatin fraction [1] is enriched

with nonhistone proteins and newly synthesized RNA. On the other hand, DNA replication evidently begins with active genes [7], but DNA replication in fact begins in the chromatin fraction firmly bound to the matrix [2, 5], which is rich in unique sequences [2]. Comparison of the present results with the facts described above suggests that the chromatin fraction firmly bound to the matrix is enriched with actively transcribable genes.

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UREA SYNTHESIS IN HEART MUSCLE

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Urea formation by the isolated rat heart was studied during perfusion with NH_4Cl (10 mM), mixtures of NH_4Cl (10 mM) and L-aspartic acid (10 mM) and L-ornithine (2.5 mM) with L-arginine (10 mM), L-glutamine (10 mM), L-alanine (10 mM), L-leucine (5 mM), and pyruvate (5 mM). The most effective activator of urea synthesis is NH_4Cl and L-arginine; L-leucine and pyruvate have an inhibitory action. It was shown with the aid of the isotope ^{15}N that ammonia fixation can take place in the heart tissue through the formation of urea. The quantity of ^{15}N incorporated into urea increases with an increase in the concentration of ammonium-^{15}N acetate in the perfusion fluid from 1.6 to 3.4 mM. Isoproterenol necrosis of heart muscle leads to a significant increase in ^{15}N incorporation into urea.

KEY WORDS: urea; synthesis; ammonia, amino acids.

Information in the recent literature on the concentration of urea in the heart muscle and on its role in cardiac activity is extremely limited. Most conclusions of the urea balance in the heart have been based on the study of the coronary arteriovenous difference [2-4, 10]. The following rule was observed in this case: In the presence of high concentrations of urea in coronary blood (> 1 mM) it was assimilated by the heart, whereas in the presence of low concentrations it was eliminated. Hence it can be concluded that the exchange of urea between the blood and heart is determined by gradient.

On the other hand, there is evidence that urea can be formed in the heart itself [4, 13]; activity of enzymes of the ornithine cycle (arginase, orthithine carbamoyltransferase, arginine-succinase) has been found in homogenates of the heart of man and animals [1, 2, 14, 15].

The connection of urea with metabolism of other nitrogen-containing compounds in the heart has been studied in [4, 5]. Investigation of nitrogen metabolism in a group of patients with ischemic heart disease showed

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