DENATURATION OF MOUSE SATELLITE AND RIBOSOMAL DNA DURING HYDROXYAPATITE THERMAL CHROMATOGRAPHY OF CHROMATIN

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Abstract. Mouse DNA and chromatin were melted on hydroxyapatite and the denaturation profiles of ribosomal and satellite DNAs were followed by hybridization with their complementary RNAs. Neither ribosomal nor bulk DNA had significantly different melting profiles in chromatin as compared to DNA. However, most of satellite DNA eluted at higher temperature from chromatin than from purified DNA. One explanation for the higher melting temperature of mouse satellite DNA in chromatin suggests that the complex between this particular DNA component and at least some proteins in chromatin is more stable than the average DNA-protein interaction.

I. INTRODUCTION

Hydroxyapatite thermal chromatography has been used by McConaughy and McCarthy for fractionation of chicken erythrocyte chromatin [1]. These authors have found that when melted in solution this chromatin showed a much smaller low-melting component than liver chromatin and interpreted this in favor of the hypothesis that transcribed DNA sequences are contained in the low-melting chromatin. They showed that the low-melting chromatin fraction, separated by hydroxyapatite thermal chromatography, contained those DNA sequences complementary to red cell RNA. Recently, however, Reeck failed to detect any difference in the amount of low-melting DNA after denaturation in solution of liver and erythrocyte chromatin [2]. On the other hand, in our preliminary experiments on hydroxyapatite thermal chromatography of chromatin and DNA, almost identical melting curves and T_m values were found for both chromatin and DNA, unlike the results obtained after melting in solution. The question to be answered is: do the identical melting profiles obtained under the conditions of hydroxyapatite thermal chromatography reflect an identical way of melting both DNA and chromatin (as far as determined DNA sequences were concerned) or does the chromatin structure affect the denaturation of DNA in the

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nucleoprotein. One approach to answer this question is to study the melting profile of known DNA sequences in purified DNA and in chromatin after thermal denaturation on hydroxyapatite. Mouse satellite and ribosomal DNAs were chosen since they represent extreme DNA components both in base composition and transcriptional activity in the cell. It was found that the thermal stability of mouse satellite DNA was different in purified DNA and in chromatin.

II. MATERIALS AND METHODS

1. Isolation of chromatin and DNA
Chromatin from mouse liver (Inbred strain C57BL/6J) was isolated according to McConaughy and McCarthy [1]. To obtain DNA, mouse liver nuclei were lysed with 1% SDS and digested with 1 mg/ml of pronase overnight at 37°C. After phenol extraction the aqueous layer was dialysed either against 0.01 M borate buffer (pH 9.2) for gradient centrifugation in Cs$_2$SO$_4$ – Ag$^+$ or against 0.12 M phosphate buffer (pH 6.8) for hydroxyapatite column chromatography. [$^3$H]-labeled mouse DNA was isolated from mouse ascites tumor cells. DNA from fractionated chromatin was isolated by incubating the fraction with SDS (1%) and pronase (100 $\mu$g/ml) at 37°C for 4 hr, followed by phenol extraction and dialysis against 0.1 x SET (SET is 0.15 M NaCl, 0.05 M Tris and 0.005 M EDTA, pH 8.0). DNA and chromatin were sonicated for 40 sec at 0°C in a Branson Sonifier Cell Disrupter (Model W140).

2. Purification of mouse satellite DNA
Mouse satellite DNA was purified from bulk DNA by two successive Cs$_2$SO$_4$ – Ag$^+$ buoyant density gradient centrifugation steps [3] in a 50.1 fixed angle Spinco rotor, at 35 000 rev/min for 48 hours. All fractions containing satellite DNA were pooled and dialysed against NaCl to remove silver. The purity of the satellite DNA was checked by centrifugation in a neutral CsCl gradient in a Model E Analytical centrifuge. Light and heavy strands of satellite DNA were separated by alkaline gradient centrifugation in CsCl containing 5 mM EDTA and 1 mM K$_3$PO$_4$ in a fixed angle 40 rotor at 33 000 rev/min for 48 hours.

3. Purification of Xenopus laevis ribosomal DNA
Amplified ribosomal DNA was isolated from ovaries of animals 6 to 8 weeks after metamorphosis [4].

4. In Vitro RNA synthesis
Complementary RNA was synthesized with *E. coli* RNA polymerase using mouse satellite L-strand DNA and Xenopus laevis native ribosomal DNA as templates. Sequence homology between Xenopus and mouse ribosomal DNA was established by Sinclair and Brown [5]. The reaction conditions were identical to those reported by Reeder and Brown [6]. *E. coli* RNA polymerase was the gift of R. Reeder.