DISTRIBUTION OF SATELLITE DNA IN MOUSE LIVER CHROMATIN FRACTIONATED BY ECTHAM-CELLULOSE CHROMATOGRAPHY

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SUMMARY: When mouse liver chromatin is fractionated on ECTHAM-cellulose columns, the earliest-eluted fractions, those which are highly repressed *in vitro*, contain increased amounts of satellite DNA, averaging 18% in several preparations. In contrast, the latest-eluted fractions, those which are transcribed nearly 50% as well as protein-free DNA by *E. coli* RNA polymerase *in vitro*, contain much less satellite DNA than whole chromatin, about 3%.

INTRODUCTION

Constitutive heterochromatin, occuring at equivalent sites of homologous chromosomes and remaining heteropycnotic throughout the cell cycle, is genetically inactive and may well serve a structural role in the chromosome. Such chromatin contains a high content of highly repetitious DNA, and in organisms where satellite DNA's have been detected, they appear to be localized in such heterochromatin. Comings has pointed out the importance of distinguishing this constantly heteropycnotic chromatin from the reversible heteropycnosis associated with reversible genetic inactivation, as in mature lymphocytes [1]. While the latter material shares the characteristics of nontranscription and cytologic condensation with true constitutive heterochromatin, it can be transformed into euchromatin, *e.g.* during blast formation induced by lectins, while constitutive heterochromatin (at least the α subclass) has never been reported to lose its heteropycnotic character [7]. A number of reports have appeared concerning the partial purification of constitutive heterochromatin by differential sedimentation [2-6].

We have recently studied a different approach to chromatin fractionation, using ion-exchange chromatography on a weak cationic adsorbent, ECTHAM-cellulose, to separate repressed and transcribable segments of sonicated chromatin. Compo-

sitional, structural and functional features of chromatin fractions eluting in various positions from the adsorbent have been detailed [7-12]. In this note, I report the distribution of satellite DNA in fractionated chromatin from mouse liver. The earliest fractions eluted from ECTHAM-cellulose contain nearly 20% of their DNA as satellite sequences, renaturing at a C_0t of 1-2 x 10^{-2} mole liter^{-1} sec while the last eluted fractions, those which are readily transcribable *in vitro*, essentially lack satellite DNA (*ca.* 3% of DNA sequences).

MATERIALS AND METHODS

Chromatin was isolated from liver of male Balb/c mice, sonicated and fractionated on ECTHAM-cellulose exactly as previously described for rabbit liver chromatin [7]. Samples were dissolved in 1 M $NaClO_4$ and extracted twice with chloroform-isoanyl alcohol (27:1), precipitated with two volumes of absolute ethanol at -20° for over 16 hr and pelleted. After dissolution in 15 mM NaCl, 1.5 mM sodium citrate, pH 7.0, samples were digested sequentially with ribonuclease A (Worthington Corp., DNase free, 50 µg/ml, 37°, 4 hr) and autolyzed pronase (Sigma B grade, 100 µg/ml, 37°, 6 hr). After a further solvent extraction the purified DNA was alcohol-precipitated and dried.

Sonication is a mandatory prerequisite to fractionation of chromatin on ECTHAM-cellulose. As the size of the DNA in these chromatin segments is of the order of 1000 base pairs, direct determination of satellite content by banding the nucleic acid in isopycnic gradients is precluded. Therefore, DNA was dissociated and re-annealed prior to banding to (A) increase the density of non-reannealed unique and moderately repetitive sequences and (B) create higher molecular weight interstrand networks for the reannealed samples, facilitating their narrow banding. DNA was therefore dissolved in either 15 mM NaCl, 1.5 mM Na-citrate, pH 7.0, or 0.12 M phosphate buffer, equimolar mono-and dibasic sodium phosphate (PB*), at a concentration of 20 µg/ml, dissociated at 100° for 5 min, allowed to reanneal at 60° and then quenched in an ice-water bath. In some instances, the reannealed double-stranded DNA was separated from the remainder of the nucleic acid by hydroxyapatite

* PB - equimolar monobasic and dibasic sodium phosphate buffer.

chromatography. A 1 gm hydroxyapatite column (BioRad HTP) in 0.12 M PB at 60° was employed for 80 µg of DNA. After dilution in 4 ml of 0.12 M PB, the sample was loaded, the column washed with 0.12 M PB to elute single-stranded DNA and then with 0.5 M PB for elution of double-stranded DNA. The latter fraction was dialyzed to water and lyophilized. Column eluates were monitored in a Beckman DB-G spectrophotometer equipped with a log recorder and flow cell of 1 cm path length. Constant flows were obtained by pumping the columns, enabling calculation of reannealing by integration of the recorder plots, assuming that the single stranded fraction had a hyperchromicity of 1.4.

Analytical ultracentrifugation in 56% (w/w) optical grade CsCl (Research Plus Laboratories, Denville, N.J.) was performed in a Beckman Model E Ultracentrifuge equipped with monochromator, ultraviolet scanner and multiplexer accessories using an AnGTi rotor. Samples were sedimented to equilibrium (36-48 hr) at 20-22° and 44,000 rpm. *Micrococcus luteus* DNA, ρ = 1.731, was used as a reference. Densities were calculated as described [13] both from the known initial concentration of CsCl in the samples and from the position of the reference bacterial DNA and the values agreed within 0.005 gm/cm^3 in all cases.

RESULTS

Chromatography of sonicated chromatin on ECTHAM-cellulose leads to a broad featureless elution profile. A number of properties vary systematically across the elution profile, suggesting that under this envelope there are a series of nucleoprotein types differing in composition, structure and function [7-12]. The fraction of rapidly annealing DNA sequences (defined as annealed at C_0t = 1-2 x 10^{-2} mole liter^{-1} sec) varies strikingly across the elution profile (Figure 1). The earliest eluting fractions have nearly 20% of their DNA sequences in this class, the fractions in the middle range have a lower content which gradually decreases to approach zero in the latest eluted chromatin segments. These late-eluted segments are those which have been shown to be transcribed nearly 50% as well as protein-free DNA by *E. coli* RNA polymerase *in vitro* [12]. The rapidly reannealing sequences thus appear to be concentrated in chromatin segments which are highly re-

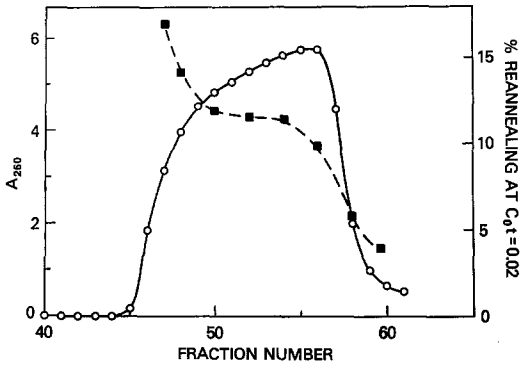

Figure 1: Content of rapidly reannealing DNA sequences in chromatin fractions derived by ECTHAM-cellulose chromatography. Chromatin was fractionated on ECTHAM-cellulose as described in Methods. The distribution of DNA is indicated by absorbancy at 260 nm (o—o—o). After isolation of DNA from selected fractions, the nucleic acid was dissociated, reannealed to a C_0t of 0.02 mole liter^{-1} sec and the extent of reassociation determined by hydroxyapatite chromatography. The proportion of DNA reannealed is plotted as a percent of the total (■- -■—■).

pressed and lacking in transcribable segments.

Analytical isopycnic banding demonstrates that the rapidly reannealing DNA sequences are indeed satellite DNA. Denatured, non-annealed DNA bands at a density of 1.715. Satellite DNA when present bands at its normal double-stranded DNA density 1.690. I examined the pellet which is obtained after shearing crude chromatin to ensure that satellite DNA was not preferentially localized in this fraction which is normally discarded in our chromatin preparation. Additionally, reannealed DNA from whole sonicated chromatin and early-and late-eluted ECTHAM-cellulose fractions was sedimented. The pellet contains satellite DNA in an amount of about 8% of the total nucleic acid (Figure 2). The content of satellite in whole chromatin is about 9%, in respectable agreement with the results of others [14] (Figure 2). Early chromatin has an augmented content of satellite, averaging anout 18% in several preparations, one of which is illustrated (Figure 3). Only a suggestion of satellite DNA is seen in the late-eluted ECTHAM-cellulose sample (Figure 3).

Several independent preparations of mouse liver chromatin were analyzed by isopycnic banding of early and late chromatin DNA, as tabulated in Table I. The range

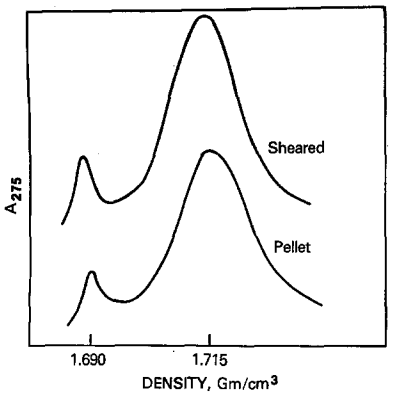

Figure 2: Isopycnic sedimentation of DNA from whole sheared chromatin and the pellet remaining after shearing, as indicated. DNA was isolated from whole sheared mouse liver chromatin and from the pellet which sediments (5000 xg, 5°, 15 min) after shearing (Virtis "45" homogenizer, 90 volts, 90 sec, 1 mM Tris-Cl, pH 8.0) in our routine chromatin preparation scheme. After dissociation and reannealing to C_0t = 0.01 mole liter^{-1} sec, the DNA was banded in 56% CsCl.

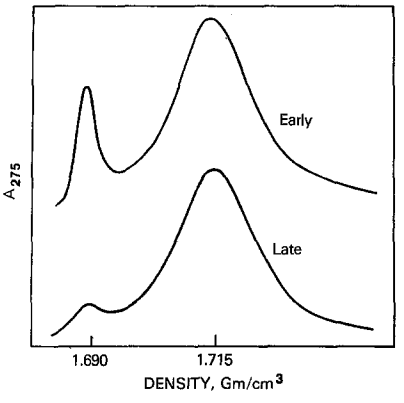

Figure 3: Isopycnic sedimentation of DNA from early-and late-eluted ECTHAM-cellulose fractions of mouse liver chromatin, as indicated. Early-eluted chromatin derives from the first 15% of the eluate while late-eluted chromatin is from the last 15% of the material eluted. DNA was isolated, reannealed to C_0t = 0.01 mole liter^{-1} sec and banded in 56% CsCl.

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of satellite in early DNA is 15-22%, with an average of 18.2%. In contrast, late eluted chromatin has a satellite content ranging from 2-5% of the total, averaging 3%. The satellite proportion of satellite DNA in the two chromatin fractions satellite distribution differs by 4.25 to over 7 fold in the five cases examined.

TABLE I

Satellite DNA in ECTHAM-cellulose Chromatin Fractions

Preparation Number	Satellite DNA as % of Total DNA in	
	Early	Late
1	22	5
2	15	2
3	19	3
4	18	3
5	17	4

Early fractions derive from the first 15% of the total column eluate while late fractions are from the last 15% of the eluted material. DNA preparation, reannealing and isopycnic sedimentation were carried out as described in the Methods Section.

DISCUSSION

The distribution of satellite DNA reflects the distribution of constitutive heterochromatin [1-6]. When chromatin is fractionated on ECTHAM-cellulose it is apparent that the earliest-eluted material is enriched in its content of constitutive heterochromatin. Comings and Mattocia, in an extensive attempt to purify chromatin containing only satellite DNA, were able to achieve about 50% of the DNA sequences in their best sample as satellite (4), suggesting that constitutive heterochromatin might constitute as much as 40% of the initial chromatin eluted from the column. These fractions are highly repressed for *in vitro* transcription by *E. coli* RNA polymerase [12], have a highly condensed structure [10,11] and contain more histone than the bulk of chromatin [8], in some cases. Such functional, structural and compositional features are those which might have been expected for genetically inactive heterochromatin.

Also as expected, the content of satellite DNA in the late-eluted transcribable chromatin is small. Since samples were pooled for DNA preparation and isopycnic banding, the average value of 3% is an upper limit for satellite DNA content in the latest-eluted chromatin. These segments of chromatin are those which may be genetically expressed *in vivo* and the lack of constitutive heterochromatin in their makeup is appropriate for this function. This is the first examined chromatin fractionation scheme not based on differential velocity sedimentation for which differences in satellite DNA content among the various fractions has been demonstrated (*c.f.* 15).

It is now apparent that at least three species of chromatin segments with varying degrees of cross contamination exist in the elution envelope from chromatography on ECTHAM-cellulose. Initially, constititive heterochromatin elutes, followed by euchromatin which is repressed *in vitro*, and then, at the tail of the elution, by chromatin which is transcriptionally active *in vitro*. Demonstration of the presence of heterochromatin in the early-eluting material is of importance in interpretation of current studies of the subunit structure of these fractions and hybridization studies aimed at elucidating functional *in vivo* differences between the three moieties.

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