Rat hepatoma cells nucleolar DNA. II. A possible model of nucleolar DNA organisation

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ABSTRACT

A model of nucleolar DNA organization has been established. Three clearly defined main components are found in ascites hepatoma cell nucleolar DNA by CsCl gradient analysis. A linear arrangement for nucleolar DNA and a model of DNA organization in the neighbourhood of a set of ribosomal genes, which may play a fundamental role in the elaboration of nucleolar chromatin tertiary structure, are presented.

INTRODUCTION

In order to elucidate the relations between structure and function of chromatin in eukaryotes, it is necessary to determine DNA organization, on which interactions between DNA and proteins involved in structure and transcriptional regulation processes depend. Many studies have been carried out using physicochemical¹⁻⁴ and biochemical techniques (for review see⁵⁻⁷). The fundamental questions about the properties of DNA (complexity, repetitious sequences...) have been raised and formulated as models of chromatin organization.⁸⁻¹⁴

To approach these problems, it is fundamental to try to isolate a definite part of chromatin containing DNA in which there are genes coding for easily characterizable RNAs. It appeared that the DNA associated with the nucleolus, from which the part containing ribosomal genes (rDNA: 1 to 2% of total nucleolar DNA) has been extensively studied by Birnstiel et al¹⁵ and Miller et al¹⁶, could meet this requirement. Furthermore, during the isolation, nucleolar chromatin retains most of those characteristics that it possesses "in situ" (compactness, staining...), unlike chromatin when isolated from nucleus, whatever the means of its preparation. In the analysis of nucleolar DNA the following elements have been particularly studied: complexity, base composition, presence of reiterated sequences, hybridization with ribosomal RNA, existence of zones with a destabilized secondary structure. In accordance with the experimental data, models of a

linear organization of nucleolar DNA, and of nucleolar DNA in chromatin, which take into account the existence and properties of two patterns of nucleolar chromatin in situ, are put forward in this paper. Only the results which justify the proposed models are presented here. An extended description of experiments will be found in separate publications¹⁷⁻²¹.

EXPERIMENTAL DATA

In the Zajdela hepatoma ascitic cells, 1.3 pg of DNA (10% of total DNA) can be isolated in association with the nucleolar structure. It is known that the genes coding for rRNA are transcribed in the nucleolus²²; 0.61% of nucleolar DNA hybridizes with 45S RNA, and 45% of this with the ribosomal RNAs (28S +18S). If we take the restricting hypothesis that the genes coding for the precursor of rRNA are the only ones to be transcribed within the nucleolus and that the low molecular weight RNAs contained in the nucleolus are synthesized elsewhere, the ratio of total nucleolar DNA to transcribed nucleolar DNA is 160 (highest value). Furthermore it has been shown that ribosomal cistrons are separated by untranscribed base sequences, spacers,¹⁵ which, in HeLa cells, are nearly as long as ribosomal genes²³. Supposing that these sequences represent an amount of DNA equal to rDNA, the excess of nucleolar DNA of unknown function is then no more than 80. Therefore it is possible to isolate a DNA fraction containing a definite gene and a degree of complexity lower than that of total DNA²⁴.

In table 1, the experimental results obtained using various techniques of DNA analysis have been summarized. DNA extracted from purified nucleoli bands homogeneously in CsCl with a density of 1.700¹⁹. After sonication of isolated DNA, three main components are found. Their density in CsCl are respectively: 1.707, 1.700, and 1.690¹⁷. Ribosomal cistrons are mostly localized in a fraction whose buoyant density is greater or equal to 1.707. It must be pointed out that some of the ribosomal cistrons show a density of 1.725, which is the theoretical density expected for ribosomal cistrons calculated from the 45S RNA base composition value. This suggests that clusters of rDNA¹⁵ (density 1.725) are inserted in stretches of nucleolar DNA composed of different kinds of segments.

A fraction of nucleolar DNA, devoid of repetitions sequences, shows a secondary destabilized structure which has been shown by freezing a DNA solution in presence of sodium p-aminosalicylate^{19,20}. Such

DNA is obtained, after freezing, in a single stranded form in an amount that varies from 0 to 50% according to the experimental conditions. 80% and 20% of this DNA possess respectively in a native form buoyant densities of 1.700 and 1.690. It is interesting to note that, between the limits of 15% and 50% of DNA in a single stranded form, a constant amount of rDNA is found. Two kinds of reiterated sequences with a medium reiteration degree make up each for 7.5% of total DNA. and possess a complexity of 10^8 and 10^9 nucleotide pairs respectively as determined by measurement of C_{ot} . One of these sequences is found in higher proportions in the nucleolus than in the total nucleus.

Table 1.

Different properties of nucleolar DNA fractions.

Buoyant densities	1.725- 1.717	1.707	1.700	1.690
Percentage of total DNA	<0.5%	24%	51%	25%
rDNA percentage	>10%	0.55%	0.4%	<0.2%
Repetitious sequences - percentage	-	0 to 15%	0	15 to 0%
Sequences with a destabilized secondary structure.	-	-	+++	+

Zajdela hepatoma cells are labeled 4h "in vitro" with thymidine 3H (24 Ci/mmele). Nucleoli are isolated (19) and DNA extracted by Kirby's procedure at low temperature without PAS (22). After RNase treatment and purification by hydroxylapatite chromatography, nucleolar DNA is submitted to different analyses:

- Fractionation by neutral CsCl centrifugation
- Determination on each fraction of rDNA by hybridization with rRNA according to Kourilsky's procedure (28).
- Detection of repetitive sequences by renaturation at 60°C in 1xSSC
- Determination, on total nucleolar DNA, of percentage destabilized secondary structure DNA by freezing at -20°C with 6% sodium p-aminosalicylate and fractionation of single stranded DNA by hydroxylapatite chromatography, (19). Then the buoyant density of each fraction is determined.

SCHEME OF LINEAR ORGANIZATION FOR NUCLEOLAR DNA IN CHROMATIN.

Given, on the one hand, the method of disruption of DNA into fractions in which certain characteristic properties emerge, and, on the other hand, the likelihood of overlapping of different regions, we shall consider, for the elaboration of a model, that, in nucleolar DNA, three main kinds of segments (I, II, III) can be distinguished and that these

segments are characterized by the essential properties of the fractions described above. The basic defining features of these segments are proposed as follows: a) Definition of classes I, II, III.

The nucleolar DNA classes are defined on the following hypotheses: - Fraction I contains all the DNA of buoyant density 1.707, all the ribosomal cistrons, and all the sequences with a medium degree of reiteration.

- Fraction II contains 50% of the nucleolar DNA including all the DNA of a buoyant density of 1.700, and consequently the sequences which respond to the action of PAS.

- Fraction III contains the DNA of buoyant density 1.690 and the sequences with a high degree of reiteration.

In classes I and III, the reiterative sequences, which are able to renature quickly, would limit the process of DNA melting brought on by PAS.

Classes I, II, III are linked together in a linear arrangement, assuming that nucleolar DNA arising from chromosomal nucleolar organizer forms a single stretch. According to our experimental data, their pattern would be: II-III-I-III-II.

All these hypotheses are summarized in Table II.

b) Organization of the primary structure of nucleolar DNA in the neighbourhood of a set of ribosomal genes.

Table II.

Definition of I, II, III nucleolar DNA fractions.

	I	II	III
Percentage of total DNA	25%	50%	25%
Buoyant densities	$\rho = 1.707$	$\rho = 1.700$	$\rho = 1.690$
rDNA percentage in relation to total nucleolar rDNA	100%	0	0
rDNA percentage in relation to total DNA of the fraction (x2, to take account of spacers)	5%	0	0
Reiterated sequences (10 ⁸)			
Percentage in relation to total nucleolar DNA	100%	0	0
Percentage of fraction's DNA	30%	0	0
Less reiterated sequences (10 ⁸)			
Percentage in relation to total nucleolar DNA	0	0	100%
Percentage of fraction's DNA	0	0	30%
DNA segments susceptible to PAS action	0	100%	0
Uncharacterized DNA	65%	100%	70%

Basic features that define the three nucleolar DNA classes by their essential properties.

Figure 1.

Proposed linear arrangement of nucleolar DNA.

Figure I accounts for the principles presented above. A set of ribosomal genes is made up of sequences coding for the 45S RNA, separated by non-transcribed sequences (spacers); the whole of these sequences could represent a part of rDNA, as described by Birnstiel et al.¹⁵. This set of genes would, for example, be headed and followed by highly reiterative nucleotide sequences which might play a role in the regulation of its transcription. These sequences could either correspond to the sites of recognition by RNA polymerase A²⁵, or represent an element implicated in the regulation of rDNA transcription. The sum of these sequences compose fraction I. On each side of these groups are found DNA, which form fraction III containing sequences with a medium degree of reiteration, that could play a role in the elaboration of the tertiary structure of nucleolar chromatin (see below). In the presented model, the distribution of reiterated sequences in one or both of classes I and III is not fundamental since the essential facts are the separation of the PAS-sensitive fractions and non-PAS-sensitive ones, and the vicinity of fractions I and III.

The fractions III-I-III are linked to the remaining DNA (fraction II) which contains the regions possessing a secondary structure sensitive to the action of PAS. The buoyant density of such a fraction (III-I-III) is close to that of bulk DNA and can be indistinguishable from it before fractionation of DNA by sonication. Yet experimental data¹⁷ suggest that III-I-III segments are truly bound to fraction I, and that all kinds of fragments (I, II, III) are colinear in the stretches of DNA containing rDNA clusters.

Such an organization model of nucleolar DNA might be applied to

any DNA sequence containing one or several genes. However, for a non-redundant gene or a relatively short polycistronic sequence, the various elements which are parts of this model, would not exist in great enough amounts to be shown up by this technical approach

c) Model of organization for DNA in nucleolar chromatin.

Nucleolar DNA is distributed "in situ" into two types of chromatin, the intranucleolar and perinucleolar chromatin. The transcription of 45S RNA occurs at the level of intranucleolar chromatin²¹. Furthermore, we have established²¹ some correlations between the biosynthesis of 45S RNA, partial inhibition of protein synthesis and the presence of intranucleolar chromatin. (Inhibition of rRNA biosynthesis corresponds to a retraction of intranucleolar chromatin.) The proposed model takes these facts into account, and the actively transcribed rDNA is represented as a part of the intranucleolar chromatin.

The condensed nucleolar chromatin appears for the most part super-helical²⁶. The DNA regions with a secondary destabilized structure would be localized at the folding loci of the super helix which would induce a partial destacking of the bases, while the DNA would assume an intermediate canonical form.

Fraction 111 is found in greater proportions in the nucleolar chromatin than in total nuclear chromatin, and could play a role in the formation of intranucleolar ramifications from the perinucleolar chromatin. Through folding of the DNA molecule, two regions of chromatin containing fraction 111 get in touch and build a super helix.

Non-histone proteins with a specific role would interact with these sequences and induce the formation of this particular structure. These proteins would be thermolabile and could be renewed quickly: thus, through the effect of supranormal temperature or protein synthesis inhibitors, reorganization of nucleolar chromatin occurs, involving the disappearance of intranucleolar chromatin^{21,26}. This phenomenon could also be the result of modifications of the histone by acetylation which induces chromatin condensation or decondensation.

Fraction 1, which is included between two fractions of type 111 is localized in a loop-shaped chromatin structure which constitutes the intranucleolar chromatin. The adjacent type 11 fractions which remain at the nucleolar periphery could be linked to the whole of perinucleolar chromatin.

Figure 2.

A) Linear arrangement of nucleolar DNA in the neighbourhood of a set of ribosomal genes.

B) Tertiary structure of DNA in perinucleolar and intranucleolar chromatin.

Figure 2 summarizes the various parts of this model and nucleolar chromatin structural conformation.

In conclusion, we have tried to demonstrate, by means of these mostly hypothetical models, that the nucleotide sequences in DNA, or better its primary structure, play an essential role in the elaboration of the tertiary structure of chromatin. This agrees with Bram's hypothesis¹⁴. Moreover, on account of its primary structure, the nucleolar DNA would have the potentiality to constitute a tertiary loop-structure which might be the site of 45S RNA biosynthesis and of its maturation. The modifications of this structure could be the key to the regulation of rDNA transcription. Nucleolus formation would result from the coalescence of a certain number of similar structures.

These presented models are essentially working hypotheses. Some recent experiments on nucleolar chromatin fractionation are in good agreement with them. In another part of our work in this field we are trying to test the veracity of the proposed model of DNA nucleoli organisation in other cell sublines.

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