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STRUCTURAL HETEROGENEITY OF CHROMATIN PREPARATIONS AT THE LEVEL OF DNA TOPOLOGY

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The view that genomes of eukaryotic cells contain circular supercoiled DNA domains has been confirmed in the last decade [7, 9]. Chromatin preparations, which are model systems of the eukaryotic genetic apparatus, are convenient objects with which to study the topology of the genome under normal and pathological conditions, although they have not yet been used for this purpose. The view that domains with circular DNA ought to be preserved, at least partially, in chromatin preparations [2] was confirmed by the present writers by rheologic analysis of the effect of the dye ethidium bromide (EB), intercalating into DNA, on chromatin in a deproteinizing solution [3].

The aim of this investigation was to study the structural heterogeneity of chromatin preparations at the level of DNA topology, using the method of chromatin fractionation [1], by means of which chromatin fibrils containing linear and circular DNA could be separated.

EXPERIMENTAL METHOD

Chromatin was obtained from calf thymus by washing the tissue homogenate 4 times in 0.025M Na_2—EDTA + 0.075 M NaCl (pH 8.0) to remove ribonucleoproteins. The chromatin residue was washed once in a mixture of 0.15M NaCl + 0.7 mM Na-phosphate buffer (pH 7.0) and was dispersed in the same medium and homogenized for 50 and 100 sec, equal samples of the chromatin suspension being withdrawn at intervals for analysis. The chromatin preparations (C_{DNA} = 200 μg/ml) were transferred into 0.7 M NaCl in Na-phosphate buffer, with the addition of an equal volume of 1.25 M NaCl into Na-phosphate buffer. The solution was poured into a test tube (50 cm^3, internal diameter 28 mm) and a glass rod (diameter 5 mm), revolved by an electric motor at a speed of 60 rpm for 90 min, was lowered into it coaxially. Under these circumstances some of the chromatin was adsorbed on the rod and removed together with it from the solution. Solutions of unadsorbed chromatin and also the initial chromatin sus-

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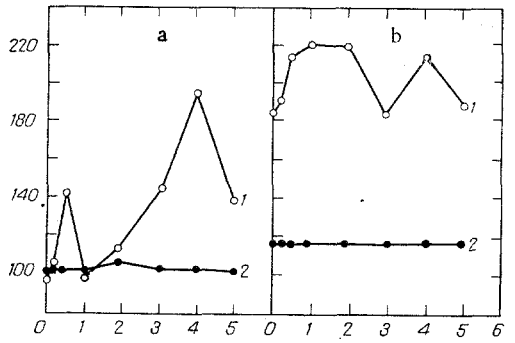

Fig. 1

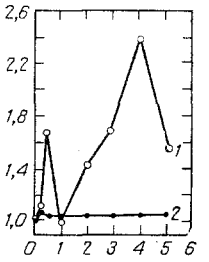

Fig. 2

Fig. 1. Dependence of reduced viscosity of calf thymus chromatin solutions in deproteinizing medium on EB concentration. Abscissa, EB concentration (in μg/ml); ordinate, reduced viscosity η_{sp}/C_{DNA} (in dl/g). a) Preparation No. 1; b) preparation No. 6. 1) Total chromatin preparations; 2) unadsorbed chromatin fractions.

Fig. 2. Effect of EB on viscosity of chromatin in deproteinizing medium before and after additional mercaptoethanol. Abscissa, EB concentration (in μg/ml); ordinate, ratio of reduced viscosity of chromatin solution in the presence of EB to corresponding value in absence of EB. 1) Chromatin; 2) chromatin + 5% mercaptoethanol.

pensions, were transferred into deproteinizing medium, used to produce cell lysis [5]: 1 M NaCl + 0.1 M Na_2-EDTA + 1 mM Tris + 0.5% Triton X-100, and adjustment to pH 8.0 with NaOH. The viscosity of the resulting solutions was measured on a rotary viscometer with sheer stress of $4.5 \cdot 10^{-3}$ dynes/cm^2 in the presence of various concentrations of EB. Concentrations of DNA in the chromatin preparations and of EB were determined as described previously [3].

EXPERIMENTAL RESULTS

As a result of chromatin fractionation (C_{DNA} = 100 μg/ml), as described above, in 0.7 M NaCl the quantity of DNA in the fraction unadsorbed on the revolving rod, in different preparations in the order of conduct of the experiments, was 32, 8, 3, 33, 19, 33, and 36%. Dependence of the reduced viscosity of chromatin preparations No. 1 and No. 6 (curves 1) and of their unadsorbed fractions (curves 2) in deproteinizing medium at C_{DNA} = 10 μg/ml on the EB concentration is shown in Fig. 1. These dependences are depicted by curves with two maxima in the case of both total chromatin preparations. These maxima were absent when unadsorbed fractions of both preparations were analyzed.

The presence of maxima on the curves in Fig. 1 can be taken to be associated with the existence of supercoiled circular (covalently closed) DNA molecules or of circular (closed into rings by the residual protein contained on DNA in a deproteinizing medium) DNA molecules [5, 8]. The increase in viscosity was due to a decrease in the degree of negative supercoiling of the circular DNA on account of intercalation of EB into the DNA, and the decrease in viscosity was due to subsequent positive supercoiling of these DNA domains [8]. The presence of two maxima may reflect the structural heterogeneity of supercoiled circular DNA (sccDNA) in the chromatin preparations (possibly due to a difference in the original degree of negative supercoiling). The absence of extrema on the graphs of dependence of viscosity of the solutions of the unadsorbed chromatin fractions on EB concentration is evidence of the absence of sccDNA in these fractions. By using this method it was thus possible to separate chromatin fractions containing circular and linear DNA, because of differences in their adsorption properties.

These differences were unconnected with histone H1 and the majority of nonhistone proteins of the chromatin (in particular, with HMG proteins), which were dissociated in 0.7 M NaCl. There are no grounds for attributing adsorption of the chromatin fraction with sccDNA on account of histones of the nucleosomal nuclei, which are accessible for competing macromolecules only in chromatin fibrils with linear DNA, whereas in fibrils with sccDNA they are

TABLE 1. Characteristics of Chromatin Depending on Duration of Homogenization in 0.15 M NaCl + 0.7 mM Na-Phosphate Buffer (pH 7.0)

No. of chromatin preparation	Duration of homogenization, sec	DNA content in unadsorbed chromatin in 0.7 M NaCl, %	Reduced viscosity of objects in deproteinizing medium (C_{DNA} = 10 μg/ml), dl/g	
			total chromatin	unadsorbed chromatin fraction
4	0	33	176	46
	50	42	77	64
	100	44	64	51
5	0	19	272	84
	50	26	211	109
	100	30	204	112

safely hidden by DNA coiled on protein octamers [2]. It is logical to suggest that adsorption of chromatin fibrils with sccDNA is due to nuclear matrix proteins, firmly bound with DNA, which are absent in linear fibrils or are so reorganized that the fibrils are no longer capable of adsorption. The possibility cannot be ruled out that in the fraction of adsorbed chromatin there are fibrils with linearized DNA, containing fragments associated with protein of the nuclear matrix. The quantity of DNA in the unadsorbed chromatin fraction can therefore by regarded as an exact estimate or an underestimate of the fraction of linearized DNA in the chromatin preparation.

It will be clear from Table 1 that an increase in the duration of homogenization of the chromatin preparations increased the contribution of the unadsorbed fraction with linearized DNA and also increased the viscosity of this fraction in deproteinizing medium. The entrance of linearized sccDNA was small and the effect of degradation of previously linearized DNA predominated. The viscosity of total chromatin, on the other hand, steadily decreased in deproteinizing medium in the course of its homogenization, and it did so, moreover, even if the viscosity of the unadsorbed fraction increased. This is evidence against the presence of a significant proportion of linearized sccDNA bound with nuclear matrix proteins in the adsorbed fraction, which could lead to an increase in viscosity of the total deproteinized chromatin. The fall in the viscosity of total chromatin during homogenization is difficult to understand if it is assumed that the chromatin preparations contained only individual sccDNA domains, for their degradation, accompanied by linearization of DNA ought to lead to an increase in viscosity, as may be seen in the case of the unadsorbed chromatin fraction. The viscosity of total chromatin evidently decreased during homogenization on account of degradation of the subunits of the nuclear matrix present in it, and which, in the modern view [4], are rosette-like particles in the form of protein globules with several attached loops of sccDNA.

The role of proteins in maintenance of the sccDNA domains in chromatin is illustrated by Fig. 2. Under the influence of 5% mercaptoethanol the extremal character of dependence of the viscosity of the chromatin solution in the dissociating medium on EB concentration disappeared, i.e., rupture of disulfide bonds in the residual protein preserved on DNA in the dissociating medium led to linearization of sccDNA.

To estimate the dimensions of the DNA in the chromatin preparations, we determined the molecular weights of the isolated DNA preparations ($25 \cdot 10^6$-$65 \cdot 10^6$ daltons; 38-98 kb), and also the characteristic viscosity of the chromatin preparation No. 7 in 0.15 M NaCl + 1% Na dodecylsulfate. The molecular weight of DNA in the chromatin was determined by the formula of Crothers and Zimm [6]. Its value (somewhat underestimated under these conditions) was found to be $1.1 \cdot 10^8$ daltons (167 kb) for the original chromatin, and $5.8 \cdot 10^7$ daltons (88 kb) after homogenization for 100 sec. Under these circumstances the contribution of DNA to the unadsorbed fractions containing degraded linearized DNA was 36 and 49%, respectively. Considering that the mean size of the DNA loops in the genetic apparatus of the cell is 53 kb [7, 9], it can be concluded that the chromatin preparations contain subunits of the nuclear matrix with a set of several loops or domains of sccDNA.

It was interesting to analyze the distribution of individual active and repressed genes in chromatin fractions with linear and sccDNA, for the decondensed state of the euchromatin

suggests that it was more susceptible than heterochromatin to mechanical degradation. If this reasonably evident hypothesis is confirmed, the technique we have developed will provide the research worker with fractions of weakly fragmented domains of euchromatin and complete circular domains of heterochromatin. The importance of the study of these chromatin fractions for biology and medicine is undisputed, especially in the light of the idea [10] that different kinds of physiological and pathological processes are based on differential gene expression controlled by endogenous and exogenous factors.

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COMPARATIVE FLUORESCENCE STUDY OF CELL MEMBRANES AND RECONSTITUTED LIPOSOMES

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The essential role of the plasma membranes of cells in various functional and pathological cell processes, and also in the development of responses to external injurious influences is the factor which determines the importance of the development of new and promising approaches to their study. One such approach is the use of proteoliposomes, reconstituted from solubilized cell membranes as an experimental model, and their use to modify the cell surface.

The aim of this investigation was to compare the properties of membranes from thymus lymphocytes and Ehrlich's ascites carcinoma (EAC) cells and those of liposomes reconstituted from them. The liposomes were characterized by the use of fluorescence methods, yielding quantitative information on the state of the test object while inflicting minimal damage on it during the experiments [4]. The intrinsic ultraviolet fluorescence (UVF) of the cells characterizes the quantity and state of the cell membrane proteins. The nonpolar fluorescent probe pyrene, localized in the hydrophobic part of the lipid bilayer, can be used to assess the state of the membrane lipids [2].

EXPERIMENTAL METHOD

Thymus lymphocytes were isolated from noninbred albino rats weighing 120-130 g by the method in [3]. EAC cells, transplantable into noninbred albino mice, were isolated on the 7th day after inoculation. The characteristics of fluorescence of the cells and proteoliposomes in Krebs–Ringer solution (pH 7.3) were measured on an MP-650-40 fluorescence spectrophotometer (Hitachi, Japan). Characteristics of UVF were measured within the bounds of the linear region of dependence of UVF on concentration: with concentrations of thymocytes and EAC cells of $4 \cdot 10^6$ and $0.7 \cdot 10^6$/ml, respectively, and of proteoliposomes equivalent to 0.1 μmoles of phospholipids in 1 ml. Fluorescence of membrane-bound pyrene was measured in the presence of pyrene in concentrations of 3 μmoles/ml, thymocytes $20 \cdot 10^6$/ml, EAC cells $3 \cdot 10^6$/

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