cytologic phenomenon. Further investigations are needed to explain how this phenomenon is linked with the basic monogenic defect.

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# DYNAMIC AND STATIC CHROMATIN


V. D. Paponov, G. A. Sigora, S. P. Rad'ko, and V. N. Lystsov



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KEY WORDS: chromatin; superstranded DNA; DNA — histone interaction; ethidium bromide.


In papers published in May, 1984, by Worcel and co-workers [8, 13] the concept of the existence of two types of chromatin — dynamic and static — in cells of eukaryotes was introduced. In the same month the present writers published their own data [3], which also suggested the existence of the same two types or two states of chromatin [3]. The grounds for introduction of this concept by ourselves and by Worcel's group were different, but they are mutually complementary and provide a firmer basis for views on the dynamic nature of active and potentially active chromatin and the static nature of transcriptionally inactive chromatin [3, 4, 8, 13].

In our opinion, the most important criterion for differentiation between dynamic and static chromatin is the character of relations between their main components — DNA and histone, which can easily be tested by the presence of absence of exchange of chromatin histones with free histones. Some preparations of chromatin did not show this kind of exchange, even if histone was added to them in a 200-fold excess. After ultrasonic treatment of chromatin the fraction of histones H2A + H2B, exchanged with the more competitive H3 + H4, increased, and exchange was recorded when total histone was added in only a fivefold excess [3, 4]. This weakening of the DNA-histone bond cannot be explained by a decrease in the fraction of masked (inaccessible for free histones) nucleosomes due to destruction of interfibrillary junctions in chromatin (an increase in the degree of dispersion of chromatin), for histone H1 is completely displaced in all chromatin preparations if the ratio between added histone to chromatin DNA is more than unity. This proved that all nucleosomes are accessible and that in each of them histone H1 interacts dynamically with DNA. We postulated that static relations of histones H2A + H2B with DNA are determined by the presence of fibrils with circular superstranded DNA (cssDNA) [3, 4] in chromatin preparations, in which the dynamics of the nucleosomal nuclei is limited (fluctuation unwinding), and histone exchange is thereby blocked. Linearization of cssDNA by ultrasound ought to facilitate the dynamics of nucleosomal nuclei and the dynamics of relations between their histones and DNA.

To test the hypothesis, in the investigation described below the possibility that cssDNA may be present in chromatin preparations was studied, using the phenomenon of extremal dependence of viscosity of solutions of covalently closed circular superstranded DNA on concentration of the intercalator [9, 12], which is exhibited also in cell lysates [1, 10] containing cssDNA in nuclear nucleoids not mechanically destroyed, as the criterion.

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Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. Moscow Engineering Physics Institute.


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## EXPERIMENTAL METHOD

Chromatin was obtained from calf thymus by fivefold washing of the tissue homogenate to remove components soluble in 0.025 M $Na_2EDTA$ + 0.075 M NaCl (pH 8.0). The chromatin residue, after the last washing, was dispersed in 0.15 M NaCl + 0.7 mM Na-phosphate buffer (pH 7.0) and diluted until the DNA concentraion in the suspension was 20 μg/ml. The chromatin suspension was degraded in an MSE ultrasonic disintegrator, set at high amplitude 4. Ultrasonic treatment lasted 1 and 15 min. The suspension was then treated with an equal volume of a solution of 2 M NaCl + 0.2 M $Na_2EDTA$ + 4 mM Tris + 1% Triton X-100, adjusted to pH 8.0 with NaOH. The final deproteinizing medium was similar to the mixture used by Cook and Brazell [7] to produce lysis of cells when detecting superhelical DNA formation by sedimentation of cell lysates in the presence of ethidium bromide — EtBr (1 M NaCl + 0.1 M EDTA + 2 mM Tris + 0.5% Triton X-100, pH 8.0). The viscosity of the chromatin solutions in the deproteinizing medium in the presence of different EtBr concentrations was measured on a rotary viscosimeter [2] with shear stress of $4.5 \cdot 10^{-3}$ dyne/cm$^2$. The DNA concentration was determined in the chromatin suspension before addition of the deproteinizing solution by the method in [5]. The concentration of EtBr (mol. wt. 394.3 daltons, from Serva, West Germany) was determined from the coefficient of extinction in water $E_{460\ nm} = 4{,}800\ M^{-1} \cdot cm^{-1}$ [9].

## EXPERIMENTAL RESULTS

Curve 1 in Fig. 1 shows dependence of the reduced viscosity of the chromatin solution ($C_{DNA}$ = 10 μg/ml) in the deproteinizing medium on EtBr concentration. The curve has two maxima: the first at $C_{EtBr}$ = 1 μg/ml, the second at $C_{EtBr}$ = 4 μg/ml. The molar ratio of EtBr to DNA nucleotides at extremal values of viscosity was 0.084 and 0.336, respectively. For the circular superstranded DNA of phage PM2 this ratio was 0.091 in the case of measurement of the viscosity of a solution containing 175 μg/ml DNA in 10 mM Tris-HCl (pH 7.4) + 75 mM NaCl [9]. Curves 2 and 3 in Fig. 1 show dependence of the reduced viscosity of the chromatin solution, treated with ultrasound for 1 and 15 min respectively before transfer into the deproteinizing medium. During a short exposure to ultrasound, its effect on chromatin was shown to be selective, with a decrease in the second maximum on the curve and its shift along the abscissa to $C_{EtBr}$ = 3 μg/ml, while the amplitude and position of the first maximum along the abscissa were unchanged. During prolonged exposure of chromatin to ultrasound, both maxima on the curve of viscosity as a function of EtBr concentration disappeared.

It is now generally agreed that the extremal dependence of viscosity of DNA solutions on the concentration of the intercalator is evidence of the presence of superstranded circular DNA molecules in the solution. It is considered that the increase in viscosity is due to a decrease in the degree of negative superspiralization. Total relaxation of circular DNA is obtained at maximal viscosity, and a subsequent decrease of viscosity is due to positive superspiralization of the circular DNA in response to an increase in the concentration of bound intercalator [9, 11]. Thus the extremal character of dependence of viscosity of chromatin solutions on the EtBr concentration in the deproteinizing medium, just as in the case of cell lysates [1, 10], unequivocally proves the presence of superstranded circular DNA molecules in the system studied. Disappearance of the maxima on the curve during ultrasonic degradation of chromatin (Figs. 1 and 2) suggests linearization of the DNA molecule in the chromatin, just as with ultrasonic treatment of DNA solutions [11].

It will be noted, however, that usually during the study of the effect of intercalators on the hydrodynamic properties of DNA-containing systems (solutions of DNA, cell lysates) only one maximum is found during measurement of viscosity [1, 9-11] and only one minimum during sedimentation analysis [7, 10]. We know of only one publication in which two minima were observed (at $C_{EtBr}$ values of 2 and 5 μg/ml) during a study of dependence of DNA sedimentation in lysates of mouse thymocytes on EtBr concentration [6]. The authors cited suggested that the cause of appearance of two extrema was the different accessibility of cssDNA domains for EtBr, although this does not explain the disagreement with the data obtained by Cook and Brazell [7], who found one extremum during analysis of other cells in the same lytic medium. The presence of two extrema cannot be explained likewise by any special features of the thymocytes, for we found similar effects of EtBr on the viscosity of human leukocytes in the same medium as that in which calf thymocyte chromatin was studied (Fig. 2).

In our view the two extrema in Figs. 1 and 2 reflect differences in the degree of DNA superspiralization, either in different cell populations (thymocytes and leukocytes constitute a heterogeneous cell population), or the same difference in domains of active and inactive chromatin of individual cells. This second hypothesis can explain the absence of a sec-

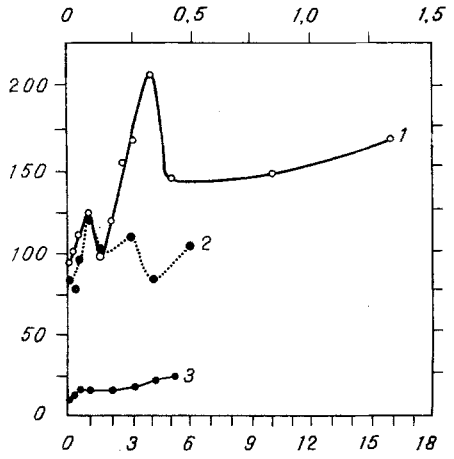


Fig. 1

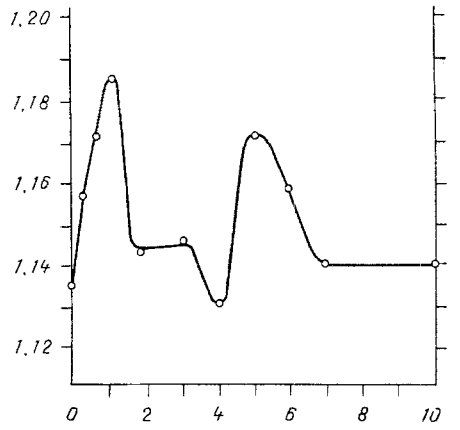


Fig. 2

Fig. 1. Dependence of reduced viscosity of calf thymus chromatin solutions in deproteinizing medium on ethidium bromide concentration. Abscissa, concentration of EtBr: below — μg/ml, above — molar ratio of EtBr to DNA nucleotides; ordinate, reduced viscosity ($\eta_{sp}/C_{DNA}$, dl/g). 1) original chromatin; 2) chromatin after ultrasonic treatment for 1 min; 3) chromatin after ultrasonic treatment for 15 min.

Fig. 2. Depedence of relative viscosity of dispersion of human leukocytes in deproteinizing medium on ethidium bromide concentration. Abscissa, concentration of EtBr (in μg/ml); ordinate, relative viscosity (η of solution/η of solvent).

ond extremum on the curve of hydrodynamic characteristics of cell lysates as a function of intercalator concentration in DNA, with increased sensitivity of cssDNA in domains of active chromatin to the DNA-relaxing agents of the cells, as has been shown for torsion stresses of SV40 minichromosomes (2-5% of the population) [12], and for half of these same plasmid minichromosomes, assembled in *Xenopus* oocytes [8, 13]. The possibility of differences in sensitivity of the cssDNA domains to mechanical degradation must be borne in mind, as is clear from the selective effect of ultrasound on the appearance of extrema in Figs. 1 and 2.

The discovery of cssDNA in chromatin preparations (Fig. 1) confirms the hypothesis that transformation of static relations of histones of nucleosomal nuclei with DNA into dynamic, in the course of ultrasonic treatment of the chromatin, is due to linearization of the cssDNA [3, 4].

These data complement views of Worcel et al. [8, 13] on dynamic and static chromatin, which implies increased ability of DNA to relax in domains of dynamic, torsion-stress chromatin, assembled with the aid of ATP-dependent topoisomerase 2. The dynamic character of histone—DNA relations in the context of their competitive displacement by macromolecules with higher affinity for DNA is extremely important for performance of RNA-polymerase transcription. However, the question of whether linearization of fibriis is essential for this dynamics or whether transitions of cssDNA domains into a special potentially active state with disintegration of nucleosomes into seminucleosomes [8, 13] is sufficient, requires further study.

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# ROLE OF DNA REPAIR PROCESSES IN SISTER CHROMATID EXCHANGE FREQUENCY CHANGES IN PERIPHERAL BLOOD LYMPHOCYTES DURING INFLAMMATORY DISEASES

N. V. Umnova, E. Yu. Moskaleva, and G. G. Poroshenko

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KEY WORDS: sister chromatid exchanges; DNA repair; inflammatory diseases of the lungs.

Since the discovery of sister chromatid exchanges (SCE) in cells [12] and the beginning of their active study with bromodeoxyuridine [7] many aspects of this phenomenon have been studied and, in particular, variability of the SCE level depending on different factors. It has been observed that the final yield of SCE is largely dependent on the characteristics of the stage of the cell cycle during exposure, the clinical nature of the induced lesion, the rate of its removal and the degree of preservation of lesions of different types, leading to the appearance of SCE [13].

DNA injuries arising spontaneously in the cell during its biological activity (apurine and apyrimidine regions, etc.) activates a number of mechanisms leading either to chromosomal aberrations or to SCE. The main role in these mechanisms is played by repair processes, aimed at removing the lesions or converting them into a less dangerous form for the cell. Changes in the intensity of these processes and, in particular, in human lymphocytes, lead to changes in the SCE level [3].

Recent data showing the higher frequency of reduced ability of lymphocytes to carry out repair in patients with nonspecific lung diseases (NSLD) and a further weakening of this ability after a course of antibiotic therapy [1] suggested that changes may also occur in parameters such as SCE.

The aim of this investigation was to study the frequency of SCE in human peripheral blood lymphocytes in patients with nonspecific inflammatory diseases of the lungs.

## EXPERIMENTAL METHOD

Patients hospitalized for NSLD (acute pneumonia, chronic bronchitis, bronchial asthma during an exacerbation) were investigated before the beginning of treatment, i.e., before administration of any therapeutic substances, and immediately after the end of antibacterial therapy (5-7 days after the beginning of administration of drugs). Healthy persons of the corresponding age and sex served as the control group.

Whole blood (0.5 ml) was cultured for 72 h in medium RPMI 1640 with the addition of L-glutamine and inactivated calf serum, in the presence of bromodeoxyuridine (10 μg/ml). Colchicine (0.5 μg/ml) was added 2 h before fixation. Hypotonic treatment was carried out in KCl solution, followed by fixation with a mixture of ethanol and acetic acid (3:1). Films were stained by the method in [2].

Ability of peripheral blood lymphocytes to repair DNA was expressed in conventional units, and was assessed as the ratio between reparative synthesis, stimulated by a standard dose of UV irradiation (100 J/m$^2$) and spontaneous reparative synthesis. An ability to repair of under 2 conventional units (c.u.) was considered to be low [1].

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