Structure of DNA in metaphase chromosomes of mouse fibroblasts

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We show that N-1 in adenine of chromosomal DNA is methylated by treatment of metaphase chromosomes with dimethylsulphate while this is not the case in chromatin. The data on methylation are consistent with those obtained from the experiments with S_1-nuclease treatment of chromatin and chromosomes. This suggests a disarrangement of DNA secondary structure in the metaphase chromosomes.

Chromosomes *Chromatin* *Methylation* *DNA structure*

1. INTRODUCTION

The data on the structure of metaphase chromosomes are contradictory. They concern mainly the higher levels of its organization, whereas the state of DNA in metaphase chromosomes remains unknown. This study was aimed at the secondary structure of DNA in the chromosomes of mouse fibroblasts. As a tool, the chemical modification of DNA with dimethylsulphate was used. The treatment of DNA with low concentrations of DMS [1] caused neither appreciable changes in its secondary structure, nor a removal of histones from DNA–histones complexes. The methylation in native DNA is most efficient at N-7 in guanine and somewhat less pronounced at N-3 in adenine. N-1 in adenine being almost unaffected. The extent of the methylation of N-7 and N-3 indicates the accessibility of major and minor grooves, while that of N-1 indicates the presence of regions with disarranged secondary structure. It was found that N-1 in adenine of chromosomal DNA is methylated by DMS treatment of metaphase chromosomes, while it remains unaffected in the chromatin from the same cell lines. The data on methylation are consistent with those obtained in the experiments with S_1-nuclease treatment of the chromatin and chromosomes. This suggests a disarrangement of DNA secondary structure in the chromosomes.

Abbreviations: DMS, dimethylsulphate; Tris, tris-hydroxymethylaminomethane; EDTA, ethylenediaminotetraacetic acid

2. MATERIALS AND METHODS

2.1. *Preparation of chromosomes and chromatin*

Chromosomes were isolated from a culture of mouse fibroblasts (line A9) presynchronized in the presence of colchicine (1 μg/ml) for 12–18 h, metaphase cells amounting up to 90–95%. The standard method of isolation was followed [2]. Nuclei and undamaged cells were separated by additional centrifugations at $1000 \times g$ for 5 min. Isolated chromosomes were examined under light microscope, the contamination with RNA was determined by the orcein reaction [3]. Chromatin from cell culture (line A9) and from rat liver (strain Wistar) was isolated as in [4]. Only fresh chromatin was used.

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2.2. *Methylation*

[^{14}C]DMS (31 Ci/mmol) was from Amersham. Methylation with DMS was done in 0.2 ml 0.02 M sodium cacodilate, 0.1 mM EDTA and 0.01 M $MgCl_2$ (pH 7.0). The final concentration of DNA was 40 A_{260} units/ml. DMS (125 μCi) was added in 0.16 ml dimethylsulphoxide. Reaction mixture was incubated at 37°C for 4 h, DNA was isolated by phenol–detergent method and precipitated with ethanol in the presence of 0.1 M NaCl. The precipitates were washed twice with ethanol, dissolved in 0.2 ml 0.01 M Tris–HCl, and subjected to RNase treatment. RNase A (10 μl, 40 units/mg Reanal) and 20 μl RNase T_1 (100 units/ml, NIKTI BAV, Novosibirsk) were added to DNA solution and the mixture was incubated at 37°C for 15 min. DNA was isolated once more by the above method. To hydrolyze DNA, the precipitates were held on boiling water bath for 60 min in the presence of 8 M $HClO_4$ and were then neutralized with 8 M KOH in the presence of phenolphtalein in a trace amount. The supernatants emerging after centrifugation at 4000 × *g* for 10 min were analyzed on column with Dowex 50 × 4 as in [1], and fractions were counted in dioxane scintillating liquor.

As a control, native and denatured DNA from calf thymus (Sigma) were methylated. DNA was denatured by heating at 100°C for 5 min followed by cooling in ice.

2.3. *S_1-nuclease treatment*

The treatment of chromatin and chromosomes with S_1-nuclease was carried out in 0.01 M sodium acetate, 0.01 M $MgCl_2$, 0.01 M $ZnSO_4$, (pH 4.6). Final concentration of DNA was 20 A_{260} units/ml for chromatin and 14 A_{260} units/ml for chromosomes. S_1-nuclease (30 000 units/ml, NIKTI BAV, Novosibirsk) was added to 750 units/ml final conc. The reaction proceeded at 37°C for 60 min and the amount of $HClO_4$-soluble products were measured as in [5].

3. RESULTS AND DISCUSSION

The results of DNA modification, chromatin and chromosomes are presented in table 1. The table shows that the extent of modifications of N-7 in guanine of chromatin and chromosomes is much the same, while the one of N-1 in adenine is significantly increased in chromosomes as compared to that in chromatin. This leads to a decrease in the ratio me^7G/me^1A from 27 in chromatin to 6.6 in chromosomes, the last value being stable in chromosomes regardless of the overall extent of the modification (fig. 1). Therefore, it seems unlikely, that the modification of N-1 adenine is just due to the admixture of the polynucleotide material with extremely high reactive adenine. The enhanced extent of modification of N-1 adenine in chromosomes may be explained if some changes in the secondary structure of DNA in chromosomes are involved. The ratio me^7G/me^1A in chromosomes reached that in the DNA in which denatured regions comprise 70% of its full length (table 1). This ratio can also be obtained providing

Table 1

The extent of modification and ratio of methylated bases in DNA, chromatin and metaphase chromosomes

Sample	mmol me^7G / mol DNA	mmol me^1A / mol DNA	me^7G / me^1A
DNA native	15.6	0.39	40
DNA denatured	16.1	7.45	2.18
Chromatin	14.6	0.54	27
Chromosomes	13.6	2.27	6.6

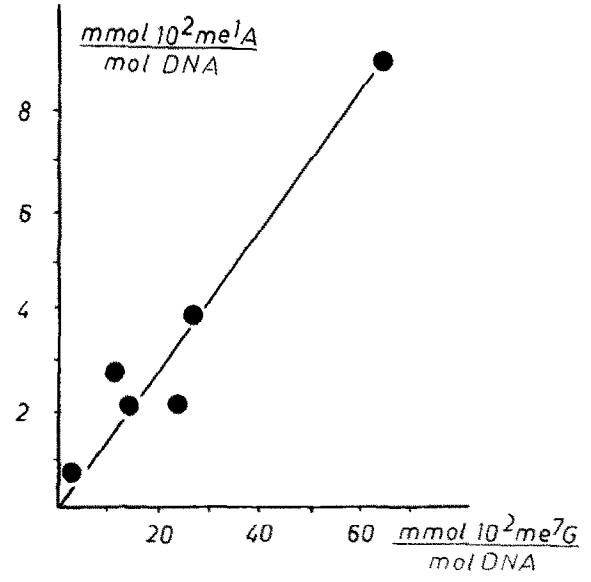

Fig. 1. Dependence of ratio me^1A/me^7G on the overall extent of methylation.

that the admixture of RNA in chromosomes constitutes up to 26 % of all nucleic material. This is not the case, since in a separate experiment (not shown) the RNase treatment resulted in a complete digestion of exogenous RNA (ribosomal RNA) added to chromosomes. The amount of poly(A) tracts which may produce the observed ratio in chromosomes can be calculated provided that DNA in the chromosomes is double-stranded throughout its length. The extent of modification of N-1 in adenine in completely denatured chromosomes is by 7.06×10^{-2} mmol/mol DNA; i.e., 28×10^{-2} mmol/mol adenine residues higher than that in the DNA isolated from chromosomes under standard conditions. The extent of methylation of N-1 in chromosomes as compared to that in chromatin is higher by 1.53×10^{-2} mmol/mol adenine residues. Thus, poly(A) tracts can constitute 5.5% of nucleotides in the chromosome preparations. However, the direct determination of the content of ribonucleotides in the preparations shows that it is $\leqslant 1\%$ of all nucleotides.

The experiments with S_1-nuclease hydrolysis gave also evidence for the appearance of abnormally structured DNA regions in metaphase chromosomes. The chromatin DNA proved to be insensitive to S_1-nuclease, while the S_1-nuclease treatment of chromosomes resulted in the release of 1.6% deoxyribonucleotide material. This figure hardly reflects a true portion of disarranged DNA regions in chromosomes, since the accessibility of such regions to S_1-nuclease is, probably, dependent on their protein environment.

The data presented lead to the conclusion that there are some regions of DNA with disarranged secondary structure in the metaphase chromosomes. One may assume that the changes in DNA structure take place in the course of the transition of chromatin into metaphase chromosomes. The events of this kind were reported earlier for the process of DNA packaging in some bacteriophages [6].

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