Differences in chromatin from normal and leukemic human cells as shown by digestion with restriction nucleases

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Restriction endonuclease *Msp*I digested significantly more than *Hpa*II the DNA and chromatin from normal and leukemic human cells, although both enzymes digested DNA more than chromatin. Moreover, DNA and chromatin from normal cells showed higher digestion by *Hpa*II compared to DNA and chromatin from leukemic cells indicating higher frequency of Cm^5CCG in latter DNA. *Eco*RII and *Bst*NI, which have the recognition sequence $CC\overset{A}{\underset{T}{}}GG$ but cut at different points, digested all DNAs significantly, as did *Bst*NI for chromatin from all sources. However, chromatin from normal cells showed only limited digestion by *Eco*RII which significantly digested chromatin from leukemic cells. This could result from subtle differences in the conformation of normal and leukemic cell chromatin involving recognition sequences for *Eco*RII.

Human leukemia *Chromatin* *DNA* *Restriction endonuclease digestion*

1. INTRODUCTION

Studies on the accessibility of DNA in chromatin to a variety of non-specific DNases have contributed to the recognition of the nucleosome model for chromatin [1]. In this model, stretches of DNA which are protected against nuclease attack by histones, seem to alternate with regions that are more easily accessible to nucleases. Additional information may possibly be obtained by analyzing the efficiency of recognizing and cleaving specific nucleotide sequences within chromatin by DNA restriction endonucleases. These site-specific DNases have been used in other studies to characterize domains within the higher order structure of chromatin [2], for the selective digestion [3], or isolation [4] of satellite DNA containing chromatin and for comparing accessibility of gene [5] which is expressed in one tissue, but not in other tissues.

Here we compared the restriction enzyme cleavage patterns of chromatin and DNA from normal and leukemic human cells. The restriction enzyme isoschizomers used have C–G in their recognition, and therefore, these studies also give information on the distribution of 5-methylcytosine in DNA, which has been proposed to be related to differentiation and gene expression [6–10].

2. MATERIALS AND METHODS

Normal lymphocytes were obtained from buffy coats by Ficoll-Hypaque centrifugation and monocytes were removed by adherence to glass. To obtain stimulated lymphocytes, the normal lymphocytes were incubated at 37°C with phytohemagglutinin-M (PHA) for 72 h. Leukemic cells from 3 acute lymphoblastic leukemia (ALL) and two acute myeloblastic leukemia (AML), patients each with >90% blasts were obtained by leukapheresis. These leukemic cells were separated further by Ficoll-Hypaque centrifugation. MOLT-4 cells, which are of thymic type ALL origin, were cultured in Roswell Park Memorial Institute 1640 medium containing 5% fetal bovine serum. These cells were harvested by centrifugation at 800 × *g*. All cells were washed

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5-times with phosphate-buffered saline before use.

The chromatin was prepared using buffers containing phenylmethylsulfonyl fluoride, and purified by ultracentrifugation at 70000 × *g*, for 2 h, through 2.2 M sucrose, as in [11]. The chromatin pellet was dispersed in 0.01 M Tris–HCl buffer (pH 7.2) and used immediately for various studies. The DNA from chromatin was prepared as in [12].

The chromatin and the DNA from normal and leukemic human cells were digested with restriction endonuclease *Hpa*II, *Msp*I, *Eco*RII, and *Bst*NI (1 unit enzyme/μg DNA for 2–4 h), using the conditions indicated by the suppliers (Bethesda Research Laboratory and New England Biolabs). All incubations were done at 37°C and terminated by adding Sarkosyl to 0.4% [13], EDTA 10 mM, sucrose 10%, and bromophenol blue 0.1%. Samples containing about 0.5–1 μg of DNA in 20 μl were loaded in slots of 1% Seakem agarose horizontal slab gels, which had been pre-electrophoresed at 100 V for 20 min. The electrophoresis was done for 4 h at 100 V at 4°C using buffer containing 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, 18 mM NaCl, and acetic acid to pH 8.4. The gels were stained with ethidium bromide (0.5 μg/ml) and photographed through a red filter, after illumination with UV light.

3. RESULTS AND DISCUSSION

The restriction endonucleases *Hpa*II and *Msp*I provide a simple, fast assay for DNA methylation [8] at the site CCGG. *Hpa*II is sensitive to methylation at the second C in its site, the most commonly methylated sequence in vertebrate DNA, whereas its isoschizomer *Msp*I is sensitive to methylation at the first C in CCGG [6–9]. *Eco*RII [8] cleaves in front of two C nucleotides in sequence ↓CC$^{A}_{T}$GG, but its isoschizomer, *Bst*NI [8] cleaved behind two C nucleotides in sequence CC↓$^{A}_{T}$GG, even if the cytosine was methylated.

Fig.1 shows the typical fragment patterns that were obtained by gel electrophoresis of DNA from

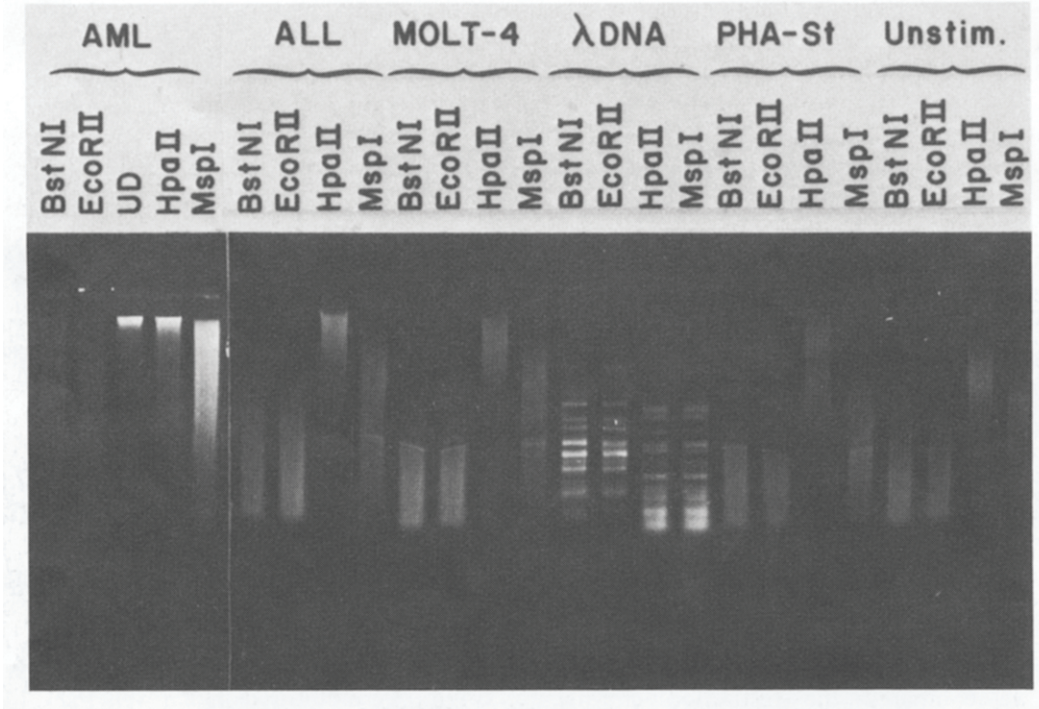

Fig. 1. Normal and leukemic human cell DNA and λDNA marker digested for 2 h at 37°C with *Bst*NI, *Eco*RII, *Hpa*II and *Msp*I (1 unit restriction endonuclease/μg DNA) and analysed on 1% agarose gels. As exemplified by AML, the undigested (UD) DNAs moved only slightly from the start for all samples examined. Unstim. and PHA-st. refer to unstimulated and PHA-stimulated normal lymphocytes. See section 2 for details.

normal and various leukemic human cells that were digested with the above restriction endonucleases. As exemplified by AML the undigested DNA from all sources migrated into the gel only slightly. Both *Bst*NI and *Eco*RII digested DNA extensively, from all sources, indicating that there was no significant methylation of CC↓$^{A}_{T}$GG sequences in these DNAs. In contrast, *Hpa*II digested, to a limited extent, all DNAs that were fragmented extensively by *Msp*I which recognizes the sequence CCGG and Cm^5CGG. Similar results have been reported for unstimulated human lymphocyte DNA [6] and mammalian DNAs [9,10,14] that were digested with *Hpa*II and *Msp*I, indicating that sequence Cm^5CGG is generally much more frequent than CCGG in mammalian DNA.

The patterns shown in fig. 1 indicate that digestion of DNA by *Hpa*II was somewhat lower for leukemic cells compared to DNA from unstimulated or PHA-stimulated normal lymphocytes. This is consistent with higher DNA methylase activity and higher 5-methyl cytosine content of DNA in leukemic cells (1.5%) compared to normal cells (1.1%) (Srivastava, B.I.S., unpublished data). Fig. 2 shows a striking resemblance between the *Hpa*II and *Msp*I digestion pattern of chromatin and that obtained with DNA (fig.1) from normal and leukemic cells. *Hpa*II showed almost no digestion of chromatin, whereas digestion of chromatin by *Msp*I was extensive, indicating the accessibility of CCGG and Cm^5CGG sequences in chromatin to *Msp*I. As found for other restriction endonucleases [15,16], the digestion of chromatin by *Msp*I and *Hpa*II, however, appears to be less than that for DNA. Again, *Hpa*II digested normal cell chromatin more than that from leukemic cells.

Molecular mass distribution patterns obtained by digestion of chromatin with *Bst*NI and *Eco*RII, fig.3, indicate that chromatin from both normal and leukemic cells was digested equally by *Bst*NI, as was the DNA (fig.1) from normal and leukemic

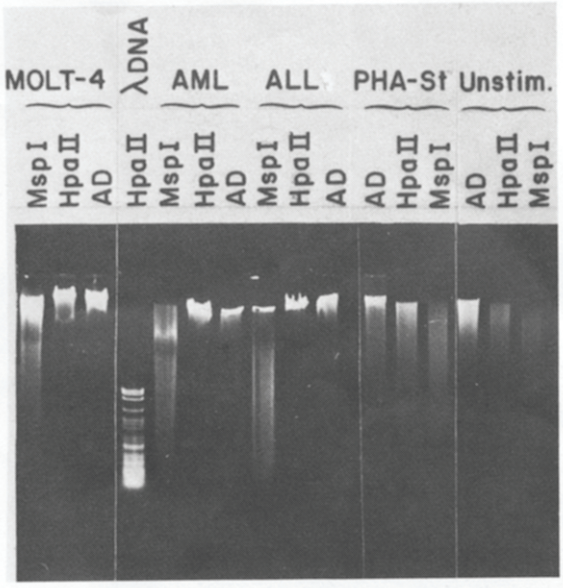

Fig. 2. Chromatin digested with *Hpa*II and *Msp*I and λDNA marker digested with *Hpa*II (1 unit restriction endonuclease/μg DNA) for 4 h at 37°C and analysed on 1% agarose gels. AD refers to autodigested chromatin without restriction endonuclease which gave the same pattern as undigested sample. Two additional ALL and one additional AML patients examined (not shown) gave patterns similar to that given for these leukemias. Rest as in fig.1.

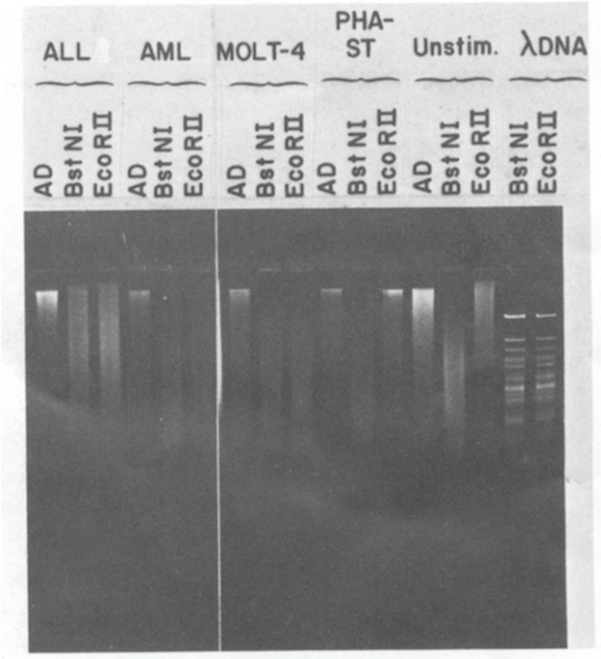

Fig. 3. Chromatin and λDNA marker digested for 4 h at 37°C with *Bst*NI and *Eco*RII (1 unit restriction endonuclease/μg DNA) and analysed on 1% agarose gels. AD refers to autodigested chromatin without restriction endonuclease which gave the same pattern as undigested sample. Rest as in fig.2.

cells. However, unlike DNA, the chromatin from unstimulated and PHA-stimulated normal lymphocytes showed only limited digestion with *Eco*RII compared to the extensive degradation of chromatin from leukemic cells by this enzyme. The chromatin from all 3 ALL and 2 AML patients as well as the chromatin from MOLT-4 cells, unstimulated, and PHA-stimulated normal lymphocytes prepared on several occasions, showed the same difference in *Eco*RII digestion of chromatin from normal and leukemic cells.

In some experiments where chromatin was prepared from isolated nuclei [17], the same results were obtained on *Eco*RII digestion as with chromatin prepared by direct isolation. Moreover, DNA from autodigested chromatin like that from undigested chromatin, showed only a slight migration into the gel, indicating no interference from any endogenous nucleases. That these differences in *Eco*RII digestion of chromatin from normal and leukemic cells were not due to the presence of inhibitors was illustrated by the normal digestion of λDNA by *Eco*RII in the presence of chromatin from unstimulated or PHA-stimulated normal lymphocytes.

Equal digestion of DNAs from normal and leukemic cells by *Eco*RII and *Bst*NI, and of chromatin by *Bst*NI, indicates that decreased digestion of normal cell chromatin compared to leukemic cell chromatin by *Eco*RII, which has the same recognition sequence as *Bst*NI but cuts at a different site, could result from subtle differences in the conformation of the chromatin involving *Eco*RII recognition sequences that makes them relatively inaccessible to *Eco*RII in chromatin from normal cells.

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