THE COMPACTION OF MOUSE HETEROCHROMATIN AS STUDIED BY NUCLEASE DIGESTION

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1. Introduction

Constitutive heterochromatin is a nearly universal feature of the eukaryotic cell nucleus (reviews [1–4]). The heterochromatic DNA consists in its majority of highly repetitive sequences [5], but the molecular basis for the highly condensed appearance of heterochromatin is not known. The compact state of heterochromatin has been exploited as the basis for its biochemical isolation. By sonication of nuclei and differential centrifugation according to [6] a rapidly sedimenting fraction could be obtained from mouse, calf, and guinea pig liver nuclei which contained mostly satellite DNA [7–9].

An alternative approach became available through the use of restriction nucleases. Differences in the susceptibility of satellite DNAs and non-repetitive DNA can be exploited for the preparation of heterochromatin by digestion of intact nuclei with restriction nucleases and subsequent extraction of chromatin ([10–14], K. v. Oefele, W. H., in preparation). It is in this case not the higher degree of compaction, however, which is the basis for isolation of the heterochromatin but instead the difference in size of the DNA. In fact, it is the characteristic of the purification scheme used subsequent to restriction nuclease digestion to decondense the chromatin as much as possible and abolish possible differences in chromatin conformation.

Here we show that mouse liver heterochromatin can be prepared as a rapidly sedimenting fraction after digestion of the nuclei under special conditions with either micrococcal nuclease or DNase II. The compact state of heterochromatin which in this case is the basis for the separation is not dependent on the continuity of the DNA but instead on non-covalent interactions which are highly sensitive to the ionic environment.

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2. Materials and methods

2.1. *Sonication and digestion of nuclei*

Nuclei were isolated as in [15]. Prior to use, they were washed 3 times in 10 mM Tris–HCl (pH 7.0), 3.3 mM $CaCl_2$ [6]. They were suspended in 0.25 M sucrose at 50 μg DNA/ml, allowed to stand on ice for 10 min and then sonicated, usually in 2–3 ml aliquots for 10 s in a Branson sonifier (S 125) using the microtip and the lowest setting. The suspension was immediately fractionated into a pellet and a supernatant fraction by centrifugation at 5000 × *g* for 10 min.

Micrococcal nuclease and DNase II were from Worthington (Freehold NJ). Digestions were done at 37°C under the conditions specified in the figure legends.

2.2. *Analytical ultracentrifugation*

DNA was isolated as in [15] and analyzed in a Beckman model E ultracentrifuge equipped with a UV scanner. DNA (2–3 μg) was centrifuged for 40–60 h at 40 000 rev./min in CsCl, initial density 1.707, buffered with 15 mM Tris–HCl (pH 8.3). Lower M_r DNA samples from nuclease-digested chromatin were centrifuged in the presence of Hoechst dye 33258 according to [16] which improves the resolution between satellite and main band DNA.

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3. Results and discussion

The classical procedure for the preparation of heterochromatin entails pretreatment of the nuclei in $CaCl_2$-containing buffer, swelling in sucrose, sonication, and differential centrifugation to obtain an insoluble heterochromatin fraction [6]. We attempted to replace sonication by nuclease treatment since sonication has been shown to harm the structure of chromatin [17], and chose micrococcal nuclease and DNase II for that purpose. After nuclease digestion at standard conditions, i.e., with micrococcal nuclease in 10 mM Tris–HCl, 1 mM $CaCl_2$, and with DNase II in 10 mM Tris–HCl [15], and subsequent extraction of the chromatin in 0.2 mM EDTA, 75–85% of the chromatin could be solubilized. However, no significant depletion or enrichment of satellite DNA occurred in any fraction. Apparently the molecular features that distinguish heterochromatin from euchromatin had been destroyed in these experiments prior to fractionation. We suspected that in some way the higher compaction of the heterochromatin might be abolished by the slight differences in ionic conditions encountered during digestion and extraction of the nuclei as compared to sonication. Alternatively it could be that the cutting patterns of the nuclease lead to the solubilization of the heterochromatin. Experiments designed to distinguish between these possibilities are described below.

We first examined the effect of ions in the preparation of heterochromatin and, in order to do that, returned to the sonication procedure. Sonication under the conditions in [6] led to a 4–5-fold enrichment of the satellite DNA in the pellet fraction (fig.1c). When the experiment was repeated under identical conditions except that 10 mM Tris–HCl was present during sonication in addition to 0.25 M sucrose a very different result was obtained. Even though ~25% of the DNA were again found in the pellet fraction there was almost no enrichment of satellite DNA (fig.1b). Instead the relative proportion of satellite DNA in the pellet and supernatant fraction was almost identical. The same result was obtained when sonication was done in 0.25 M sucrose and the Tris–HCl buffer added subsequently. This shows that the more compact structure of heterochromatin is lost upon the addition of 10 mM Tris–HCl. As expected, lower levels of Tris–HCl had a lesser effect, and <0.5 mM the heterochromatin pellet had the same composition as without Tris–HCl. Addition of 0.2 mM EDTA instead of

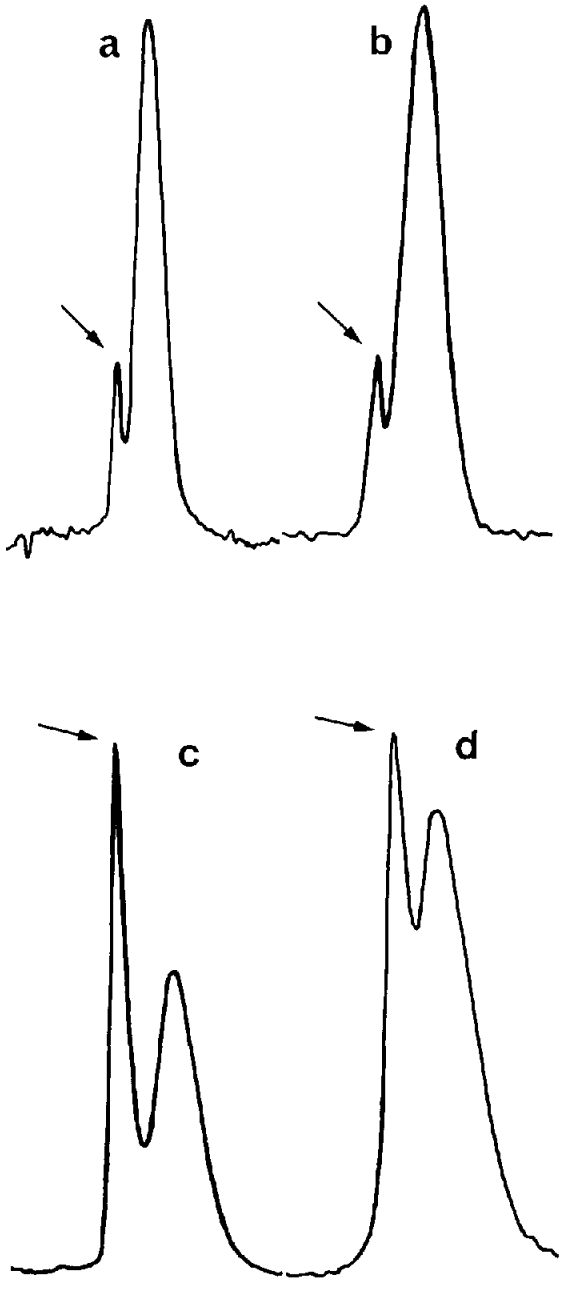

Fig.1. Satellite DNA content of different chromatin fractions. DNA was subjected to analytical CsCl gradient centrifugation. In each case the arrow designates the satellite DNA while the other peak constitutes main band DNA: (a) intact nuclei; (b) pellet fraction after sonication in 0.25 M sucrose, 10 mM Tris–HCl (pH 7.0); (c) pellet fraction after sonication in 0.25 M sucrose; (d) pellet fraction obtained after both, sonication in 0.25 M sucrose and digestion with DNase II (100 U/ml, 20 min) in 0.25 M sucrose, 0.5 mM Tris–HCl (pH 7.0). Each of the pellet fractions contained ~25% of the chromatin.

Tris–HCl had a similarly deleterious effect and prevented heterochromatin fractionation.

In contrast, digestion of the sonified nuclei in 0.25 M sucrose and 0.5 mM Tris–HCl (pH 7.0) with DNase II (fig.1d) or micrococcal nuclease (not shown) hardly interferes with heterochromatin preparation. We found that the amount of Ca^{2+} which remains bound to the chromatin after pretreatment of the nuclei is sufficient to satisfy the Ca^{2+} requirement of micrococcal nuclease. The satellite DNA content of the pellet fraction obtained after sonication and nuclease digestion was almost the same as without nuclease (fig.1) indicating that the nuclease generated cuts do not disrupt the compact structure of the heterochromatin which is the basis for its rapid rate of sedimentation.

An outcome of the above findings is that it should be possible to obtain an enrichment of heterochromatin by digesting nuclei at very low ionic strength and subsequent fractionation in the absence of EDTA. Nuclei were incubated with either micrococcal nuclease or DNase II in 0.25 M sucrose, 0.5 mM Tris–HCl (pH 7.0) the nuclear suspension fractionated into a supernatant and a pellet fraction and the pellet extracted with 0.2 mM Tris–HCl (pH 8.0). The DNA from these fractions was analyzed by agarose gel electrophoresis and, after transfer onto nitrocellulose according to [18], by hybridization to nick-translated mouse satellite DNA. For both nucleases, the DNA fragmentation patterns of the pellet and the supernatant fraction were quite similar with no major difference in the size distributions, and the satellite DNA distribution closely followed the ethidium bromide pattern. The similar accessibility of DNA in heterochromatin and euchromatin to nucleases is in agreement with [19,20]. Still, as predicted, there is a significant enrichment of the satellite DNA in the pellet fraction with a concomitant depletion in the supernatants close to what is found after sonication of the nuclei (fig.2).

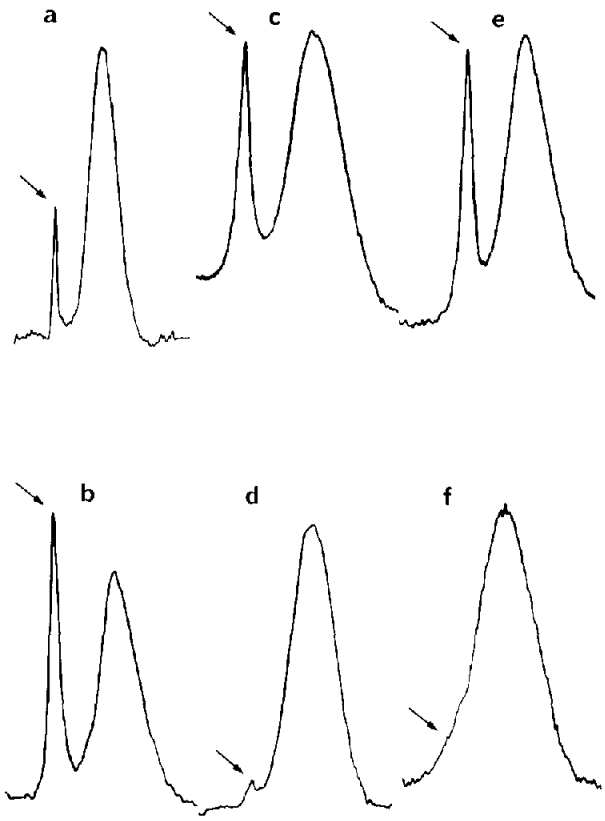

Fig.2. Heterochromatin preparation after nuclease digestion of mouse liver nuclei. Nuclei were treated with micrococcal nuclease (12.5 U/ml, 20 min) or DNase II (40 U/ml, 20 min) in 0.25 M sucrose, 0.5 mM Tris–HCl (pH 7.0). The incubation mixtures were chilled and immediately centrifuged for 10 min at 5000 × *g*. The pellets were extracted 2 times with 0.2 mM Tris–HCl (pH 8.0) and the extracted material added to the first supernatants. DNA was isolated and analyzed by analytical CsCl gradient centrifugation in the presence of the Hoechst dye 33258: (a) intact nuclei; (b) the pellet obtained after standard sonication is shown for comparison; (c) pellet; (d) supernatant from micrococcal nuclease digested nuclei; (e) pellet; (f) supernatant from DNase II digested nuclei. The arrows designate the position of the satellite DNA peak.

4. Concluding remarks

The procedure worked out for the enrichment of satellite DNA-containing chromatin is not based on the size of the DNA but instead on the more compact state of heterochromatin as compared to euchromatin. An important implication of these findings is that the features of heterochromatin which permit its isolation by biochemical methods are very sensitive to the ionic environment in a way not previously reported for chromatin. Under the conditions generally used for fractionating chromatin they are rapidly lost, and heterochromatin behaves like euchromatin. These findings provide a starting point for investigating the molecular basis of the compact structure of heterochromatin.

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