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Chromatin Degradation in Isolated Nuclei of Normal and Transformed Baby Hamster Kidney Cells[1]

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ABSTRACT

In a transformed cell line, derived from baby hamster kidney cells by treatment with ethylnitrosourea, degradation of DNA in isolated nuclei by endogenous nuclease was studied. Compared to the nontransformed cell line, the nuclear DNA of the transformed cells was found to be degraded to a much greater extent. This was reflected by a markedly lower proportion of DNA attached to the nuclear protein matrix in the transformed compared to the nontransformed cells.

These observations can be accounted for by assuming that the chromatin of the transformed cell line has a conformation different from that of the nontransformed cells.

INTRODUCTION

Residual protein structures, commonly referred to as nuclear matrices, can be isolated from nuclei of eukaryotic cells by extractions with high concentrations of salts (3, 7, 18). To these matrices, DNA was found to be attached. Depending on the conditions of isolation, the proportion of DNA bound was found to be as high as 100% (2, 7, 8). Attachment sites were shown to exist close to the replication forks transiently (4, 8, 12) and near the replication origins permanently (1, 16). It is reasonable to assume, therefore, that the attachment can be of relevance to regulatory processes or their disturbance such as in cancer cells.

Transformation of eukaryotic cells is accompanied by phenotypical changes such as loss of contact inhibition, lower serum requirement (11), and a less developed cytoskeleton (10). The nuclear matrix also seems to have been altered. Comparison of total nuclear protein from normal and transformed cells revealed the presence of polypeptides specific to each type of cell (20, 21), some of which were found to be nuclear matrix associated (20, 22).

The study which we report here is part of our effort to assess whether, due to transformation, the organization of chromatin in the cell nucleus relative to the nuclear matrix is altered. In the course of the study, it was found that chromatin of the transformed BHK[3] cells was degraded by endogenous nucleases to a much greater extent than is the chromatin of normal BHK cells. Evidence is presented for a possible change of chromatin structure as a consequence of transformation.

MATERIALS AND METHODS

Cell Culture and Isolation of Nuclei. BHK A3-cells (untransformed strain) were maintained in monolayer culture in minimal essential medium (Flow Laboratories), supplemented with 8% fetal calf serum (Grand Island Biological Co.). In this medium, BHK T33 cells (transformed strain; derived from A3 by treatment with ethylnitrosourea and subsequent selection in soft agar) were also maintained, albeit as suspension culture. Both cell strains were kindly provided by the Department of Radiation Genetics and Chemical Mutagenesis at Leiden University.

A3 and T33 cells were labeled for 3 to 5 generations with [2-^{14}C]-thymidine (0.02 μCi/ml; specific activity, 52.8 Ci/mol; Amersham) and with [*methyl*-^{3}H]thymidine (2 μCi/ml; specific activity, 20 Ci/mmol; Amersham), respectively.

A3 nuclei were isolated essentially as described before (18). Monolayers were briefly rinsed with Triton-Tris (0.1% Triton X-100 in 5 mM Tris-HCl, pH 8.0). Cells were then washed from the glass surface with an appropriate volume of Triton-Tris. Nuclei were isolated by forcing the suspension twice through a hypodermic needle (0.8 mm diameter) and centrifuging the suspension subsequently for 3 min at 1000 × *g*. The nuclei were then resuspended in 50 mM Tris-HCl, pH 8.0.

For isolation of T33 nuclei, the procedure had to be modified. T33 cells were first suspended in hypotonic medium (5 mM Tris-HCl, pH 8.0) and allowed to swell for 2 min. Then an equal volume of 0.2% Triton in 5 mM Tris-HCl, pH 8.0, was added after which isolation of the nuclei was performed as described for A3.

Mixtures of A3 and T33 nuclei were obtained by 2 alternative methods. In one, T33 cells were treated hypotonically and lysed in Triton-Tris as described above. Subsequently, the suspension was poured onto A3 cells in monolayer, and an equal volume of volume of Triton-Tris was added.

Alternatively, a suspension of A3 cells in Triton-Tris was made while T33 cells were treated hypotonically. The A3 suspension was then added to the swollen T33 cells, and immediately thereafter Triton was added to a final concentration of 0.1%.

Mixtures prepared in either of these ways were then forced through a hypodermic needle, and nuclei were collected by centrifugation. The nuclear pellet was finally resuspended in an appropriate volume of Triton-Tris.

Preparation of Lysates and Centrifugation. Either directly or after 1 h of incubation at 25°C, nuclei were collected by centrifugation. Generally, of each sample one-half of the nuclei were then resuspended in 3 ml of 50 mM Tris-HCl, pH 8.0, and 4 M NaCl was added. The NaCl lysates were subsequently layered on 2 M NaCl-containing 15 to 40% linear sucrose gradients prepared on 65% sucrose shelfs. The latter contained CsCl (0.4 g/ml). Centrifugation was performed in Beckman 27II rotors at 20,000 rpm and 20°C for 1 h.

The other half of the nuclei was resuspended in 0.5 ml 0.9% NaCl solution, to which SDS, NaOH, and EDTA were added at final concentrations of 0.5%, 0.1 M, and 0.01 M. The lysates were applied to 5 to 25% linear sucrose gradients containing 0.1 M NaOH and 0.01 M EDTA. These gradients were also prepared on 65% sucrose shelfs. Centrifugation was performed in Beckman SW 27II rotors at 17,000 rpm and 20°C for 16 h.

Fractions were collected starting from the bottom of the tube. Trichloroacetic acid-precipitable radioactivity was determined as described before (17).

RESULTS

From nuclei, obtained from both nontransformed and transformed BHK cells, nuclear matrices could be isolated by extrac-

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[3] The abbreviations used are: BHK, baby hamster kidney; SDS, sodium dodecyl sulfate.

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tion with 2 M NaCl. To analyze the DNA content of these nuclear matrices, A3 and T33 cells were labeled continuously with [^{14}C]-thymidine and [^{3}H]thymidine, respectively. Nuclear matrices were prepared and analyzed by sucrose gradient centrifugation. Results of a typical experiment are depicted in Chart 1.

The label distributions show that to the A3 matrices almost 85% of the DNA was attached (Chart 1*a*). The remainder of the DNA was found to sediment at approximately 30S, indicating that it consists of large DNA fragments most probably removed from the matrix by shear (8).

T33 matrices, however, were found to be associated with approximately 40% of total DNA only (Chart 1*b*). Moreover, the DNA detached was observed to sediment at a lower rate than the A3 DNA detached. Addition of EDTA, in mM concentrations, to the isolation media did not lead to an increase of the proportion of DNA bound to the T33 nuclear matrices.

When, in a separate experiment, equal amounts of A3 and T33 cells were mixed and extracted with 2 M NaCl, essentially similar results were obtained (Chart 1*c*). Significantly less T33 DNA was matrix attached compared to A3 DNA. Of the material detached, T33 DNA was found to have, on the average, a lower molecular weight. For the differences between the 2 cell strains, nucleolytic action was thought to be responsible.

In an additional experiment, nuclei of the same batch as used above, were incubated at 25°C for 1 h in Triton-Tris. Chart 1*d* shows that no detectable reduction of the proportion of matrix-bound A3 DNA occurred as a consequence of the incubation. Surprisingly, incubation of T33 nuclei resulted in an increase of the relative amount of DNA attached to the matrix (Chart 1*e*). However, closer analysis revealed that the absolute amount of acid-precipitable radioactivity was reduced by 75%. Therefore, the label distribution found after 1 h of incubation at 25°C was plotted relative to the distribution observed in case nuclei had been extracted with 2 M NaCl immediately after isolation. The curve then reveals a further reduction of the fraction of matrix-attached DNA. Furthermore, nearly all DNA detached was found to have been degraded to an extent either to have become acid soluble or to be able to diffuse from the nuclei.

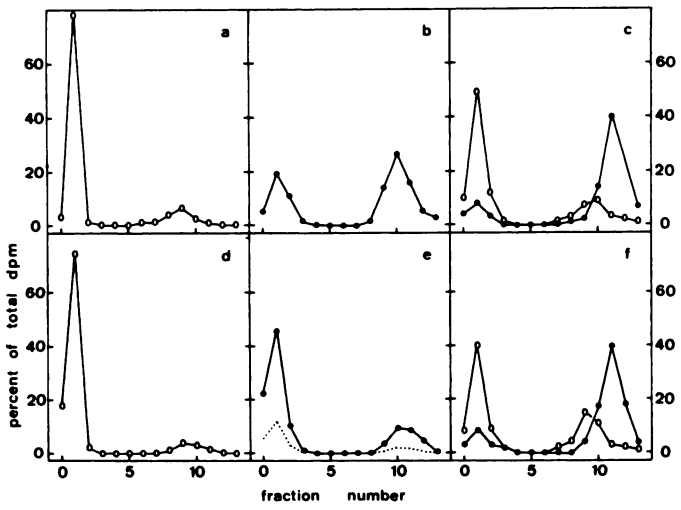

Chart 1. Analysis of DNA nuclear matrix association on neutral sucrose gradients. Nuclei were prepared from A3 cells continuously labeled with [^{14}C]thymidine and from T33 cells continuously labeled with [^{3}H]thymidine. Matrices were prepared by addition of NaCl to 2 M final concentrations either immediately (*a* to *c*) or after 1 h of incubation of 25°C (*d* to *f*). The association of nuclear DNA with the matrices was then analyzed on neutral 15 to 40% sucrose gradients. Sedimentation is from *right* to *left*. Fraction 0 represents the amount of DNA sedimented to the bottom of the tube. ○, ^{14}C-labeled A3 DNA (in dpm: *a*, 69,900; *d*, 68,700; *c*, 32,900; *f*, 34,600); ●, ^{3}H-labeled T33 DNA (in dpm: *b*, 23,000; *e*, 6,300; *c*, 46,400; *f*, 36,000). In order to compare *e* and *b*, the distribution of ^{3}H-labeled T33 DNA in *e* was also plotted on a scale similar to that of *b* (·····).

In mixtures of A3 and T33 nuclei, A3 DNA was detached from the matrix after 1 h of incubation for about 40%, an amount somewhat higher than in a separate induction (Chart 1*f*). Probably as the consequence of a dilution effect, more T33 DNA was recovered from this gradient than from a gradient containing a T33 sample only. The higher recovery is reflected mainly by an increase of the amount of DNA sedimenting at low S values (approximately 85% of total DNA). The proportion of DNA associated with the matrix after the incubation again was found to be near 15%.

Summarizing, the results of experiments with A3 and T33 nuclei separately might suggest a higher nuclease activity in T33. The data obtained from experiments in which mixtures of A3 and T33 nuclei were analyzed, however, rule out this possibility and indicate T33 chromatin might exist in a more nuclease-sensitive conformation than does A3 chromatin.

Because T33 cells are maintained in suspension culture, dead or nonviable cells, characterized by their DNA being degraded, might contaminate the samples. Consequently, DNA will be found detached from the matrix upon sucrose gradient analysis. To eliminate this trivial possibility, although not ruling it out completely, all experiments were initiated with cell suspensions of low density. All cells harvested were from log-phase cultures. Moreover, experiments yielding essentially similar results could be performed with T33 cells harvested from suspensions of different density.

Matrix instability might also be a cause of the enhanced release of T33 DNA from the nuclear matrix. To exclude this possibility, T33 cells were continuously labeled with [^{14}C]leucine in addition to [^{3}H]thymidine. Nuclei were isolated and extracted with 2 M NaCl either immediately after isolation or after 1 h of incubation at 25°C. From sucrose gradients to which the 2 samples were applied, essentially equal amounts of protein label could be recovered at positions corresponding to that of the nuclear matrix (data not shown). From these results, it was inferred that to the release of T33 DNA from the nuclear matrix matrix instability does not contribute significantly.

The degradation of both A3 and T33 DNA was examined in more detail on alkaline sucrose gradients. DNA of A3 cells lysed in alkaline SDS directly, or DNA of isolated A3 nuclei lysed in alkaline SDS immediately following isolation, sedimented for over 70% at S values equal to or higher than 70S. Upon incubation at 25°C for 1 h, some degradation was observed (Chart 2*a*).

Chart 2*b* shows that, when T33 cells had been lysed in alkaline SDS directly, a high proportion (60%) of the DNA was also found at positions corresponding to values over 70S. Nevertheless, compared to A3, the amount of DNA of lower molecular weight was found reproducibly to be somewhat elevated. Lysis of T33 nuclei immediately after isolation revealed that, in the time lapse of approximately 10 to 15 min required for nuclear isolation, nearly all DNA had been degraded to fragments sedimenting at 12S on the average. After 1 h of incubation of 25°C, the chain lengths had been even further reduced (8S), while the majority of the label either had been lost from the nuclei or had become acid soluble.

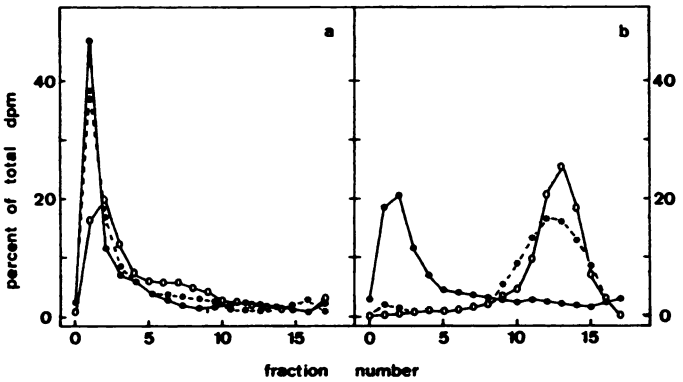

Chart 2. Analysis on alkaline sucrose gradients of the degradation of A3 and T33 chromatin by endogenous nuclease activity. A3 and T33 cells were labeled continuously with [^{14}C]- and [^{3}H]thymidine, respectively. Subsequently, either whole cells (●) were lysed in alkaline SDS, or nuclei were isolated and lysed in alkaline SDS either immediately (°) or after 1 h of incubation at 25°C (○). The lysates were analyzed on alkaline 5 to 25% sucrose gradients. *a*, A3 lysates (average total dpm per gradient, 85,000); *b*, T33 lysates (in dpm: ●, 152,700; °, 163,200; ○, 71,800).

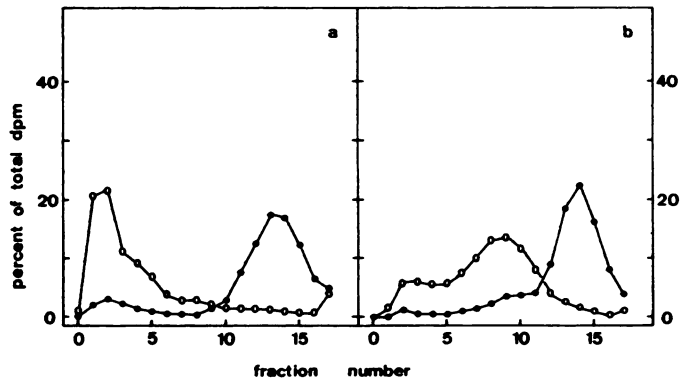

Chart 3. Analysis on alkaline sucrose gradients of the degradation of A3 and T33 chromatin by endogenous nuclease activity in mixtures of nuclei of A3 and T33. A3 and T33 cells were continuously labeled with [^{14}C]- and [^{3}H]thymidine, respectively. Nuclei were isolated, mixed, and lysed in alkaline SDS either immediately (*a*) or after 1 h of incubation at 25°C (*b*). The lysates were analyzed on alkaline 5 to 25% sucrose gradients. ○, A3-DNA (in dpm: *a*, 33,700; *b*, 37,000); ●, T33-DNA (in dpm: *a*, 40,200; *b*, 20,700).

When mixtures of A3 and T33 nuclei were lysed in alkaline SDS immediately after isolation, A3 DNA was found to be degraded only slightly, while T33 DNA was almost completely degraded to fragments of low molecular weight (Chart 3*a*). Incubation at 25°C for 1 h led to a further decrease of the length of the T33 DNA fragments. Moreover, approximately one-half of the label originally present was not recovered from the gradient. During the incubation, A3 DNA had also been nicked. The fragments generated, however, were of a significantly higher molecular weight (20 to 30S) than were the corresponding T33 fragments (6S) (Chart 3*b*). These observations could be arrived at irrespective of the way in which the nuclear mixtures had been prepared.

DISCUSSION

In the course of this study, it was found that from the nontransformed BHK strain (A3) nuclear matrices containing most of the nuclear DNA could be isolated. From matrices, obtained from the transformed BHK strain T33, however, at least one-half, and often an even greater fraction, of the DNA was lost during the isolation procedure. This difference could also be observed when matrices had been prepared from mixtures of A3 and T33 nuclei.

Sedimentation studies on alkaline gradients revealed differences in degradation of T33 chromatin compared to A3 chromatin. The most obvious cause for the differing degradation patterns is an altered chromatin structure in T33 cells acquired in the process of transformation.

In chromatin sensitivity to nucleolytic enzymes is not uniformly distributed. DNase I-sensitive domains, generally corresponding to actively transcribed genes, have been shown to exist (19). Also, nascent chromatin has a nuclease sensitivity considerably higher than that of the bulk of parental chromatin (15). The increase in sensitivity is thought to be the consequence of an altered DNA-histone interaction (9, 14).

The observed increase of the sensitivity of the chromatin of the transformed cells could be the consequence of an increase of the length of the internucleosomal linker region. Thus far, however, no gross differences in chromatin repeat length have been observed between normal and transformed cells, rendering this possibility unlikely (6, 13).

Alteration of the nuclease sensitivity of chromatin might also be brought about by the type of DNA modification (*i.e.*, alkylation) introduced by ethylnitrosourea which was used to transform the cells. However, because mutagens of this type generally introduce only a few alkylations per 10^5 base pairs (5), the increase of the nuclease sensitivity of T33 chromatin is too extensive to be accounted for by this mechanism.

On the other hand, analysis of total nuclear protein revealed differences between transformed and nontransformed cells (22). Therefore, it might be conceived that a class of protein specific to the transformed cell interacts with chromatin in a way that renders it nuclease sensitive.

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