DNA–PROTEIN INTERACTION IN HeLa CELLS IN DIFFERENT PHASES OF THE MITOTIC CYCLE AND IN RESTING CELLS

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Sharp differences were recently found in the strength of DNA–protein interactions in proliferating and resting cells by the method of nucleoprotein-celite (NPC) chromatography [2, 3, 9].

During NPC chromatography the proteins of nucleoproteins are bound irreversibly with celite and the nucleic acids are gradually liberated from the complex with proteins in a salt and urea concentration gradient, or, after maximal concentrations are reached, in a temperature gradient [1].

DNA in proliferating cells is very firmly bound with proteins (the II form) and to elute it from celite a high concentration of LiCl and urea (4 and 8 M respectively) and heating to a temperature of about 90°C are required. When the cells pass into the resting state, transition of the cell chromatin to a different type of DNA–protein interaction is observed, for which 1.5 M LiCl and 3 M urea at a temperature of 2-4°C are sufficient to produce dissociation of the complex (the I form). Transition of chromatin from the II into the I form was found to be coupled with transition of widely different types of cells from a state of division to rest.

There are two different points of view in the literature on the state of cell rest. Some workers regard it as the holding up of cells at a certain point of the cell cycle [12], others as leaving the cycle and switching of the cells into a specific resting phase (G_0) alternative to the cycle [5, 8]. Changes discovered in DNA–protein interactions during transition of cells to rest may therefore reflect either passage of the cells into the G_0 phase or arrest at a certain phase of the cycle.

To shed light on this problem the strength of DNA–protein interaction was investigated in synchronized HeLa cells at different stages of the cell cycle and in the resting place.

EXPERIMENTAL METHOD

HeLa cells were grown on medium 199 with 10% bovine serum in the presence of kanamycin (100 units/ml). The cells were labeled with [^{3}H]thymidine (5 μCi/mi) for 24 h. The cells were synchronized by the thymidine block (1.5 mg/mg, 30 h) method. Escape of the cells from the block at the boundary between the G_1 and S phases and the beginning of DNA synthesis were judged from incorporation of radioactive [^{3}H]thymidine. NPC chromatography was carried out as described in [1] and the sedimentation properties of DNA in an alkaline sucrose gradient were investigated as described in [4].

EXPERIMENTAL RESULTS

DNA of an unsynchronized culture of HeLa cells in the exponential phase of growth (3rd day after seeding, 8×10^4 cells in 1 ml), labeled for 24 h, was eluted from the column with 4 M LiCl, 8 M urea at about 90°C, i.e., in the II form (Fig. 1A, 1). Since the labeling time was about equal to the duration of the cell cycle of HeLa cells (about 24 h), DNA of cells in all phases of the cycle must have been labeled. These findings alone, namely absence of DNA in the I form, are sufficient to indicate the uniformity of DNA–protein interactions at

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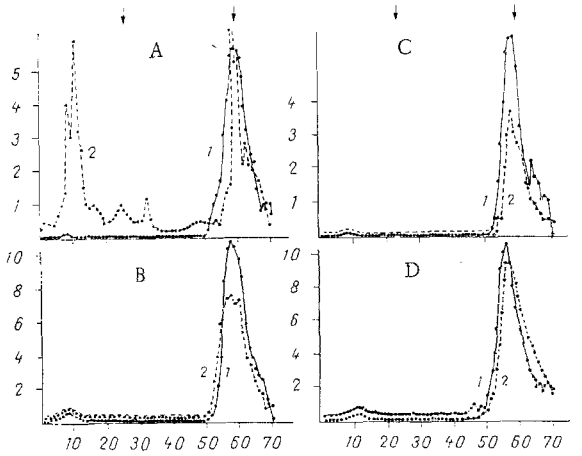

Fig. 1. NPC chromatography of DNA of HeLa cells. A) Unsynchronized cultures: 1) in exponential phase of growth, 2) in resting state; B) cells in the G_1 phase: 1) passing through the G_1 phase, 2) arrested in the G_1 phase; C) cells in the S phase: 1) passing through the S phase; 2) arrested in the S phase; D) cells in the G_1 phase (1) and cells arrested in mitosis (2). Abscissa, Nos. of fractions; ordinate, radioactivity (in cpm $\times 10^{-5}$). Arrow on left indicates beginning of temperature gradient; arrow on right indicates the 90°C point.

different phases of the mitotic cycle. However, under the experimental conditions used, some features of the DNA–protein bond characteristic of the short phases of the cycle, such as mitosis, in which a relatively small proportion of the cells (4-5%) must be at any given moment, could remain unnoticed. Accordingly, synchronized cells were used for the subsequent research.

DNA and protein in the cell chromatin in the G_1 phase (30 min after removal of the block), S phase (3 h after removal), and G_2 phase (9, 10, and 13 h after removal of the block) were found to be firmly associated (in the II form; Fig. 1:B1, C1, D1).

Even such a sharp change in the structure of the nucleus as condensation of the chromosomes in mitosis does not lead to a change in the character of the DNA–protein bond relative to the other phases of the cycle. An NPC chromatogram of the cells 17 h after replacement of the medium with 1.5 mg/ml of thymidine by fresh medium with 0.05 μg/ml of colcemid is illustrated in Fig. 1:D2. It was shown by phase-contrast microscopy that 70% of the cells in this preparation were in mitosis.

During DNA replication differences in association with proteins are possible for parental and daughter DNA strands because of binding of newly synthesized DNA with the template [10], and also because the daughter DNA strand includes a fraction of short fragments. To elucidate these possible differences, cells labeled with [^{3}H]thymidine before synchronization into the S phase were incubated for 30 min with [^{14}C]thymidine. The NPC chromatographic distribution of the ^{3}H (parental strand) and ^{14}C (daughter strand) labels was found to be identical.

Since the DNA peak on NPC chromatograms of unsynchronized cells was often asymmetrical and the position of this maximum varied during elution from 88 to 95°C, minimal differences in the character of DNA–protein interactions at different phases of the cycle were not ruled out. However, the distribution of the labels was identical on combined chromatography of cells labeled with [^{3}H]thymidine in the G_1 or in the G_2 phase on the same column with cells labeled with ^{14}C in the S phase.

It might be expected that the switch from the II into the I form would be observed when cells were arrested at any point of the cycle which, according to some workers [12], corresponds to the resting state. However, the cells preserved the type of DNA–protein interactions characteristic of cells passing through the cycle (II form) both after a 30-h thymidine block in the late G_1 phase (Fig. 1:B2) and after arrest in the S phase by cytosine arabinoside (40 g/ml, 30 min; Fig. 1:C2). The same type of DNA–protein bond in chromatin (the II form) is thus characteristic of all phases of the cell cycle, regardless of whether the cell is passing through each phase or is arrested in it.

By contrast to this the character of DNA–protein interactions changed sharply during transition of the cells into the stationary phase of growth. Cells were used in experiments on the 9th-11th day after seeding, and the medium was not changed at all throughout this period. The labeling index fell from 23 to 3%. Much of the DNA in this case changed into the I form. This transition of the cells into the resting state, but quantitatively the DNA fraction in the I form varied from 15 to 50%. These differences may reflect either a different number of resting cells in the population or differences in the "depth of rest" [5].

A similar transition also was observed when the cells were cultured for five days (from the 4th through the 8th day after seeding) in medium with the concentration of bovine serum reduced from 10 to 2%. Under these circumstances 50-70% of the DNA changed into the I form (Fig. 1:A2).

It thus becomes clear that weakening of DNA–protein interactions (the I form) reflects transition of the cells into a state qualitatively different from the mitotic cycle. The existence of DNA in the I form is a characteristic feature of the resting cell, whereas during passage of the cell through any phase of the cell cycle, and also when it is arrested in the G_1 or S phase or in mitosis, the DNA is in firm association with proteins (the II form).

Simultaneously with transition of the chromatin into the I form, accumulation of single-stranded breaks of DNA was observed in the resting cells, as other workers also have described [6, 7]. According to the results of sedimentation of DNA in an alkaline sucrose gradient the molecular weight of the single-stranded DNA fragments fell from 11×10^7 daltons for cells in the exponential phase of growth, labeled in the course of 24 h (averaged value for all phases of the cycle), to 7×10^7 daltons for cells in the stationary phase of growth.

Weakening of the DNA–protein bond is thus a characteristic feature of chromatin of the resting cell, together with an increase in the thermal stability of the DNA, lessening of its ability to bind dyes, lowering of its sensitivity to DNase I, a change in its circular dichroism spectra [8], degradation of the group of nonhistone proteins firmly bound with DNA [13], accumulation of single-stranded breaks in DNA [6, 7], and lowering of the sedimentation coefficient of the chromatin [11].

The results are evidence that qualitative differences exist in the chromatin structure of cells moving around the cycle or arrested in a particular phase of the cycle, on the one hand, and in resting cells on the other hand, and they thus confirm the view that there is a special phase (the G_0 phase) of cell rest.

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