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# REVERSIBLE ACCUMULATION OF DOUBLE- AND SINGLE-STRANDED DNA BREAKS IN DNA IN GROWTH-ARRESTED CELLS

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The formation of breaks in DNA and their repair play an important role in the regulation of many processes taking place in the cell. We know that DNA breaks accumulate during cell differentiation [10, 15], aging of cellular structures and organisms [1, 4, 14], and transition of cells into the resting state [5-7, 9, 11-13]. Stimulation of cell proliferation, on the other hand, is accompanied by repair of DNA breaks [7, 11-13]. As a rule, it is single-stranded breaks that are referred to in the publications cited above. However, detachment of DNA from the nuclear matrix in growth-arrested cells, recently discovered by the present writers, can be explained only by the formation of double-stranded DNA breaks [5].

The aim of this investigation was to study the possibility of formation and repair of double-stranded breaks in DNA during a change in the proliferative status of the cell.

## EXPERIMENTAL METHOD

Jungarian hamster fibroblasts, transformed by SV-40 virus were cultured in Carrel's flasks in a nutrient mixture containing Eagle's medium, lactalbumin hydrolysate, and bovine serum in the ratio of 4.5:4.5:1. DNA was labeled by the addition of 0.4 MBq/ml of $^3$H-thymidine to the incubation medium for 2-7 days. To arrest cell growth, the monolayer formed (7th day after seeding) was placed in medium with the serum concentration reduced to 1% and incubated under these conditions for 7-9 days. The cells were restimulated for division with fresh complete medium. The mitotic index (MI) in the exponentially growing cultures was 20‰ in resting cultures 1-2‰, and 2 days after stimulation it was 16‰. Double-stranded DNA breaks were determined by neutral elution of DNA [8] and single-stranded breaks by the alkaline DNA uncoiling method with fixation of hydroxyapatite [2].

## EXPERIMENTAL RESULTS

Typical neutral elution curves of DNA of 4/21 cells, with different proliferative status, are given in Fig. 1. DNA of actively proliferating cells has high molecular weight. During elution not more than 15% of the DNA passes through the filter (Fig. 1, 1). The character of the curves changed sharply after transition of the cells into the resting state. In some preparations the curves sloped steeply in the first fractions, but later tended asymptotically toward zero (Fig. 1, 2). Curves of the other type sloped less steeply, and about 60% of the DNA passed through the filter (Fig. 2, 1). Despite the differences between these curves, their shape is evidence of accumulation of many double breaks of DNA in growth-arrested cells. The two types of curves reflect differences in the degree of DNA fragmentation. To make sure that the accumulation of double-stranded DNA breaks is connected with a change in the proliferative status of the cell, and not with cell death, it was necessary to show that DNA fragmentation was reversible. To do this, the cells were stimulated with fresh complete medium. The neutral elution curves 48 h after stimulation were either indistinguishable from the elution curves of the exponentially growing cells (Fig. 2, 2) or sloped a little more steeply, when 20-30% of the DNA was eluted (Fig. 1, 3). Since the view was held until recently that double-

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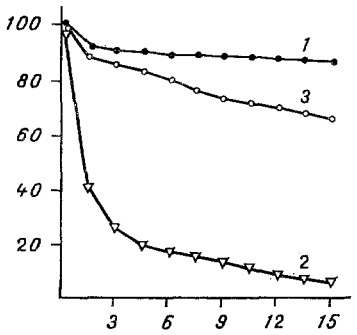


Fig. 1

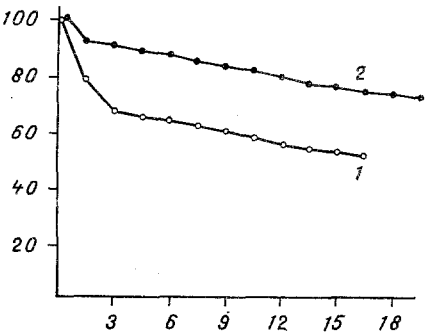


Fig. 2

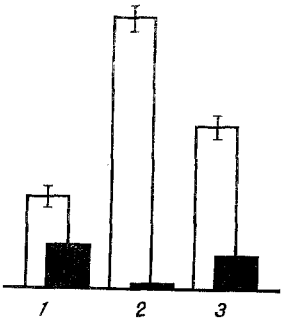


Fig. 3

Fig. 1. Neutral elution curves of DNA from 4/21 cells with different proliferative status: 1) exponentially growing cells (2nd-3rd days after seeding); 2) growth-arrested cells (incubation for 8 days in medium with 1% serum); 3) cells stimulated to proliferate (48 h after replacement of medium by complete medium). Here and in Fig. 2: abscissa, time (in h); ordinate, quantity of DNA on filter (in %).

Fig. 2. Neutral DNA elution curves of 4/21 cells at rest (1) and after stimulation (2). Cells were taken from one Carrel flask.

Fig. 3. Content of fragmented DNA (unshaded columns) and MI (black columns) of cells with different proliferative status: 1) proliferating cells (13.0 ± 3.0%, 20‰, n = 10); 2) growth-arrested cells (39.7 ± 3.5%. 2‰; n = 7); 3) restimulated cells (24.4 ± 3.6% and 16‰ respectively; n = 5).

stranded DNA breaks are irreversible, the results obtained had to be conclusively proved. To do this, some of the growth-arrested cells were removed from the glass with a scraper and used in the experiment whereas the remaining cells were added to complete medium and investigated 48 h later. Thus the same cell population was studied both at rest and after resumption of proliferation. The results of one such experiment are given in Fig. 2. After stimulation the double-stranded DNA breaks were completely repaired.

Double-stranded breaks evidently appear by addition of single-stranded. According to the results of alkaline uncoiling of DNA with fixation on hydroxyapatite, transition of cells into growth-arrested is accompanied by accumulation of many single-stranded DNA breaks (Fig. 3). Whereas the percentage of fragmented DNA in exponentially growing cells was 13.04 ± 3.00 (n = 10), on transition to the growth-arrested state the percentage of fragmented DNA increased to 39.7 ± 3.5% (n = 7), falling after restimulation to 24.4 ± 3.6% (n = 5). Consequently, an even larger number of single-stranded breaks was preserved in the stimulated cells, whereas, to judge from the neutral elution curves, double-stranded breaks were completely or mainly repaired.

Thus, the formation of double-stranded DNA breaks and their repair, in response to a change in the proliferative status of the cell, were discovered for the first time. Hitherto the presence of double-stranded breaks in growth-arrested cells has been accepted purely theoretically [7], but no direct proof was available. The increase in the sedimentation coefficients of DNA in neutral sucrose gradients during stimulation of growth-arrested cells was not considered to be significant [9]. The only physiological process during which accumulation of double-stranded DNA breaks has been found is sexual maturation in the humpback salmon. Double-stranded DNA breakds under these circumstances accumulate irreversibly in the hepatocytes [1]. The double-stranded nature of DNA breaks formed in aging cells can be deduced only indirectly on the basis of ultrastructural investigations [14]. During differentiation of Friend's erythroleukemia cells and myoblasts only single-stranded, and not double-stranded, breaks accumulate [10, 15].

The presence of an effective repair system for double-stranded DNA breaks had been conclusively demonstrated in radiobiological investigations [3], but their functional role is unclear. The breaks are perhaps necessary for loosening of the structure of certain regions of chromatin or for recombination and other structural changes to take place in the genetic apparatus of the cell, in connection with changes in its proliferative status. To solve this problem we have to discover how the breaks are distributed: randomly or in certain regions of the genome. There is as yet only indirect evidence in support of this last hypothesis [13].

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