CYTOPHOTOMETRIC STUDY OF CHANGES INDUCED BY ONCOGENIC ADENOVIRUS SA7 (C8) IN CHROMATIN STRUCTURE IN CELL NUCLEI

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Changes in the structure of nuclear chromatin of target cells under the influence of viruses have recently been reported [2, 8, 9]. It has been shown that in the early stages after virus infection changes take place in the structural organization of chromatin and are accompanied by an increase in the degree of its condensation. However, it is not yet clear whether the changes in chromatin structure take place under the influence of oncogenic adenoviruses or whether they are connected with the phase of the cell cycle.

The object of this investigation was to study the response of cell chromatin *in vitro* to infection with oncogenic adenovirus SA7 (C8) and to determine relations between the position of the cells in the mitotic cycle and the reaction of chromatin to virus infection.

EXPERIMENTAL METHOD

Primary cultures of embryonic tissue from CBA mice were grown on cover slips in medium No. 199 containing 10% embryonic calf serum. Cultures were infected with simian adenovirus SA7 (C8) in the monolayer phase (48-52 h of culture). The virus was added in a titer of log 5.4 CPD/ml. The cultures were fixed in a mixture of 100% ethanol—acetone (1:1) for 1 h, 24 h after adsorption of the virus. Control cultures were treated similarly but without infection with the virus.

The cultures were stained by the Feulgen method (hydrolysis in 5 M HCl, 37°C, 7 min, counterstaining for 45 min in a 0.5% solution of basic fuchsine, from Reanal, Hungary, pH 1.6). The cell nuclei were scanned on a Univar cytophotometer (from Reichert, Austria; planapochromat objective 100/1.32), with a circular probe 0.5 μ in diameter, and using a high-speed scanning stage (Reichert) with a 0.5 μ step. Summation and processing of the data were carried out by means of the Hewlett-Packard 9845A desk-top computer, using the WONDER program. On each side 100 cells were studied and the following parameters determined: area of the nucleus, integral optical density (IOD), and mean optical density (MOD). At each experimental point three slides (300 nuclei) were counted. On the basis of the values of IOD the cells were divided among phases of the mitotic cycle [5]. On the basis of the smooth curve of distribution of the cells by IOD and tip of the first peak of the curve (G_1) was found and taken as the mean value of IOD for cells in the G_1 phase ($\bar{x}$). On the ascending part of this curve the value of the standard deviation was found (S). The value of $2\bar{x}$ was taken as the mean value of the second peak (G_2). Virtually only cells in the S phase were found in the interval between $\bar{x} + 4S$ and $2\bar{x} - 8S$. The course of the curve in this region was continued on both sides until the intersection with $\bar{x}$ and $2\bar{x}$. The fraction of cells located in the different phases of the cell cycle was determined planimetrically) the population of S-phase cells was bounded by the segment of the curve mentioned above, the abscissa, and the $\bar{x}$ and $2\bar{x}$ axes, and the remaining parts of the first and second peaks corresponded to cells in the G_1 and G_2 phases. The distribution of optical densities also was obtained for each nucleus in histogram form, with an interval of 0.05 optical density unit (o.d.u.). On the basis of the aggregated averaged data on the frequency distribution of optical densities at each scanning point for diploid cells, curves were plotted to show dependence of the area of the points recorded during scanning on the threshold values of optical densities. The thickness of the cells on the slide was determined by means of an interference microscope on the basis of opti-

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TABLE 1. Distribution of Cells (in percent) by Phases of Cell Cycle (averaged results of 3 experiments)

Phase of cycle	Control	Cultured, infected with virus
$G_{0/1}$	57	56
S	14	21
G_2	26	20
G_2< (polyploid)	3	3

TABLE 2. Values of MOD (in o.d.u.) and Mean Areas of Nuclei (in μ^2) in Three Experiments

Parameter studied	Control			Cultures infected with virus		
	$G_{0/1}$	S	G_2	$G_{0/1}$	S	G_2
MOD	0,102±0,02	0,132±0,03	0,161±0,04	0,123±0,02	0,140±0,01	0,169±0,05
Mean area	147±2,48	212±6,4	226±5,94	124±2,57	168±1,88	195±4,36
MOD	0,092±0,01	0,114±0,02	0,128±0,03	0,108±0,02	0,128±0,02	0,146±0,03
Mean area	158±2,57	217±4,26	275±6,16	140±2,38	201±4,02	225±4,76
MOD	0,098±0,02	0,122±0,04	0,132±0,03	0,108±0,02	0,139±0,03	0,152±0,04
Mean area	151±2,62	223±7,75	256±6,56	140±2,37	207±4,76	242±5,4

Legend. Differences between experimental and control values significant by Student's t test ($P < 0.001$).

cal differences in the course of the light rays during consecutive use of two media: air and mineral oil [1].

EXPERIMENTAL RESULTS

The distribution of IOD, reflecting the relative DNA content in the nuclei, enabled the fraction of cells in the various phases of the mitotic cycle to be calculated (Table 1). Under experimental conditions after exposure to the virus marked stimulation of proliferation was observed, accompanied by an increase in the fraction of DNA-synthesizing cells by 50% compared with the control.

Data showing changes in MOD and mean area of the cell nuclei in the different phases of the cell cycle are given in Table 2 and Fig. 1. MOD of the nuclei after exposure to virus was increased on average for cells in phases $G_{0/1}$ of the cell cycle by 16.1%, for cells in the S phase by 11.3%, and for cells in the G_2 phase by 13.1%. Meanwhile the mean area of the nuclei showed a decrease of 11.5, 11.8, and 12.1% for cells in phases $G_{0/1}$, S, and G_2 respectively. Mean data on the distribution of optical densities for diploid cells in the control and after infection with virus, according to the results of three experiments, are given in Fig. 2. It will be clear from Fig. 2 that exposure to the virus led to the appearance of higher optical densities, i.e., of more highly condensed regions of chromatin, and to a shift of the histogram peak to the right.

Graphs showing the total area of the scanning points as a function of the lower threshold of optical densities recorded (Fig. 3) in control and virus-infected cultures (Fig. 3) also differed: the course of the curve was more sloping after virus infection and it ended in the zone of higher optical densities, further evidence of an increase in the degree of chromatin condensation.

Measurement of the thickness of the cells showed no statistically significant difference between the control and virus-infected cultures. Consequently, the observed increase in MOD was not the result of simple flattening of the nuclei.

The action of the oncogenic adenovirus thus leads to an increase in the degree of chromatin condensation, accompanied by an increase in MOD, a decrease in the mean values of areas of the cell nuclei, and a shift of optical densities toward higher values. These changes are characteristic of cells in different phases of the cell cycle.

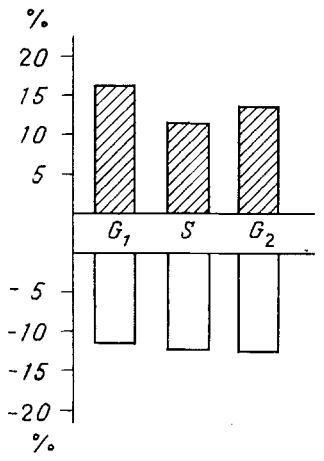

Fig. 1

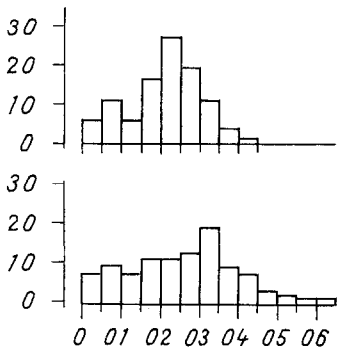

Fig. 2

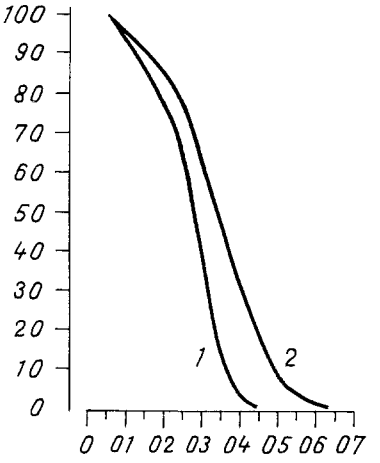

Fig. 3

Fig. 1. Changes in MOD (shaded columns) and areas of cell nuclei (unshaded columns) as a result of virus infection (mean data of three experiments).

Fig. 2. Distribution of optical densities of cell nuclei in control (top histogram) and after virus infection (bottom histogram). Abscissa, optical density (in o.d.u.); ordinate, fraction of cells in each category of histogram (in percent). Mean results of three experiments.

Fig. 3. Graph showing total area of scanning points as a function of lower threshold of optical densities recorded in control (1) and virus-infected (2) cultures. Abscissa, optical density (in o.d.u.); ordinate, changes in total area of recorded scanning points in nuclei (in percent, 100% corresponds to a 0.05 threshold).

An increase in the degree of condensation of chromatin in response to virus infection also has been found in other systems using RNA- and DNA-containing oncogenic viruses: in embryonic human lung fibroblasts infected with herpes simplex virus of types I and II [10] and with hyman cytomegalovirus [9], and in mouse lymphocytes infected with Friend's erythroleukosis virus [2]. It is not yet clear whether the increase in condensation of chromatin is connected with tumor transformation of the cells. Evidence in support of this suggestion is given by a study [8] in which a culture of strain WI-38 was compared with a line of transformed cells obtained by exposure of a culture of WI-38 to SV-40 virus: Condensation of chromatin in the transformed cells was significantly greater than in the control. However, before a final answer can be given to this question further investigations are needed.

In the investigations cited above cells were studied without regard to their position in the mitotic cycle. Our own data indicate that the increase in chromatin condensation takes place regardless of the phase of the cell cycle, although it is more marked in cells in the $G_{0/1}$ phase.

Virus infection in the present experiments stimulated proliferative activity in the culture (the fraction of cells in the S phase was 1.5 times greater than in the control). The increase in the degree of chromatin condensation under these circumstances contradicts modern views that the response of cells to stimulation toward proliferation is accompanied by decondensation of chromatin [3]. This is evidence that stimulation of proliferation under the influence of the virus differs significantly from that caused by other mitogenic factors.

It was shown previously that an increase in the degree of chromatin condensation *in situ* is accompanied by a decrease in template activity in mammalian cells not infected with virus [4, 11]. However, it was shown recently that in the early stages after infection of cell cultures with cytomegalovirus two protein factors produced in the replication cycle of the virus and associated with chromatin of the infected cells can be demonstrated [6, 7]. One of these factors leads to a change in the structure of chromatin, the other causes an increase in its template activity and is perhaps "the fast early antigen" detectable 1 h after virus infection.

The results described above are evidence of the uniqueness and complexity of responses of chromatin to infection of the cell by RNA- and DNA-containing viruses. These responses require further serious study.

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EFFECT OF PREPARATIONS OF GLUTAMIN(ASPARAGIN)ASE FROM MICROORGANISMS ON DNA SYNTHESIS IN TUMOR CELLS

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In cancer practice enzyme preparations possessing glutamin(asparagin)ase activity isolated from various microorganisms are used [1, 3-7]. However, the antiproliferative activity of preparations of this series depends on the sources from which they are obtained and the degree of purity. This paper gives comparative data on the effect of glutamin(asparagin)ase obtained from *Pseudomonas fluorescens* and *Pseudomonas boreopolis* 526 on DNA synthesis in cultures of HeLa-like human ovarian carcinoma cells (cells of line CaOv) and Fisher's lymphatic leukemia cells (line L-8).

EXPERIMENTAL METHOD

Two enzyme preparations of glutamin(asparagin)ase, isolated from different sources (the first was obtained from *Ps. fluorescens*, the second from *Ps. boreopolis* 526) and in their degree of purity were investigated. The enzyme obtained from *Ps. boreopolis* 526 was purified, as shown by disc electrophoresis, to a homogeneous state and had a specific activity of 89 I.U./mg protein as glutaminase and 71 I.U./mg protein as asparaginase. The enzyme obtained from *Ps. boreopolis* 526 was not homogeneous and had a specific activity of 40 I.U./mg protein as glutaminase and 29.5 I.U./mg protein as asparaginase. It follows from the values of specific activity given above that the enzymes differed somewhat in the ratio of their (glutaminase/asparaginase) activity: 1.25 for *Ps. fluorescens*, 1.46 for *Ps. boreopolis* 526.

The enzymes were purifed by traditional methods, which included salting out the protein with ammonium sulfate (45-90% saturation) from the protein extract, ion-exchange chromatography on DEAE-cellulose (grade DE-52), and gel-filtration. The CaOv cells were grown under standard conditions [2] in medium 199. The CaOv cells for the experiment were seeded in glass flasks (D = 2 cm) and grown for 24 h at 37°C. Each sample contained 200×10^3 to 300×10^3

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