DNA REPAIR ON THE NUCLEAR MATRIX AFTER DAMAGE BY BENZ(a)PYRENE

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Much attention has been paid in recent years to a possible association of synthetic processes involving DNA as the template with the nuclear matrix. Evidence is accumulating that DNA replication takes place on the nuclear matrix [3, 11*]. Transcription also is evidently associated with the matrix [5]. However, it is not yet clear whether attachment of DNA regions to the matrix is essential for repair of its injuries. For instance, the first attempts to analyze the possibility of association of the repair of UV-induced damage to DNA with the nuclear matrix led to contradictory results [7, 8].

The aim of this investigation was to compare the character of repair of DNA, damaged by benz(a)pyrene, in four nuclear fractions: isolated after treatment of nuclei with nuclease, subsequently extracted in buffer with low ionic strength, then extracted in 2 M NaCl, and the residual fraction with the nuclear matrix. Among these fractions two differ the most: that extracted in buffer with low ionic strength and that remaining associated with the nuclear matrix. They differ in the size of their DNA, protein composition, ratio of active to inactive genes, and degree of interaction with benz(a)pyrene [1]. The fraction extracted in 2 M NaCl is a combination of these two fractions, according to the above-mentioned characteristics, whereas the fraction extracted after mild nuclease treatment is evidently rich in DNA regions most accessible for nuclease attack.

The present investigation showed that during repair of DNA damaged by benz(a)pyrene, ^{3}H-thymidine is incorporated initially into DNA of the nuclear matrix fraction, after which its concentration in this fraction begins to fall, whereas it rises in the others. These results could indicate the need for the DNA region, damaged by benz(a)pyrene, to attach itself to the nuclear matrix for repair, and, on its completion, to revert to a position not bound with the matrix.

EXPERIMENTAL METHOD

A cell culture was prepared from 11-13-day golden hamster embryos and grown to confluence in DMEM medium (Dulbecco's modification of Eagle's medium), with the addition of embryonic calf serum to 10% and of 4×10^{-3} μCi/ml of ^{14}C-thymidine (60 mCi/mmole). The confluent culture was treated with ^{3}H-benz(a)pyrene (0.16 μg/ml, 12 Ci/mmole) for 24 h. The medium was then replaced by fresh medium not containing benz(a)pyrene or serum. The cells were harvested after different time intervals (24-72 h).

In another series of experiments unlabeled benz(a)pyrene was used in the same concentration as above, but after the medium had been replaced by fresh, ^{3}H-thymidine was added up to 0.48 μCi/ml (25 Ci/mmole). In experiments with hydroxyurea, it was added up to 5 mM during the change of medium 2-19 h before addition of ^{3}H-thymidine and was present throughout the period of incorporation of ^{3}H-thymidine.

The nuclei were purified by washing in 0.5% Triton X-100 and fractionated after treatment with restriction endonuclease HaeIII or micrococcal nuclease: 1) into a fraction extracted after treatment of the nuclei with nucleases; 2) a fraction soluble in TM-buffer with

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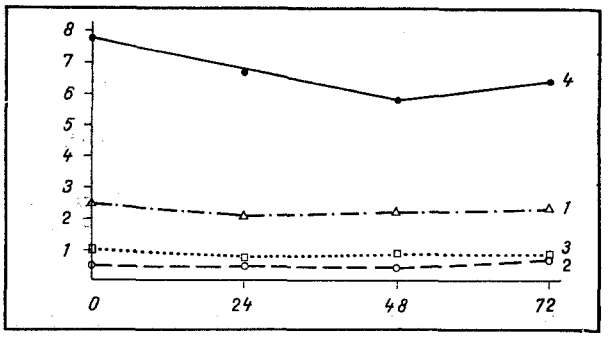

Fig. 1. Dependence of relative concentration of benz(a)pyrene metabolites covalently bound with DNA in nuclear fractions on time of repair. Abscissa, time of repair of benz(a)pyrene-damaged DNA (in h); ordinate, ratio of concentration of ^{3}H-benz(a)pyrene metabolites in DNA of above-mentioned fraction to concentration of metabolites in total nuclear DNA. DNA was isolated by the phenolic method with treatment by proteinase K and RNase, with washing in ethanol and acetone to remove covalently unbound metabolites of the carcinogen. Here and in Fig. 2: 1) DNA fraction extracted from nuclei during treatment with restriction endonuclease HaeIII, 2) DNA fraction extracted by TM-buffer with low ionic strength, 3) DNA fraction extracted with 2 M NaCl, 4) DNA fraction remaining with nuclear matrix.

low ionic strength (10 mM Tris-HCl, pH 7.6, 0.2 mM $MgCl_2$); 3) a fraction soluble in TM-buffer with 2 M NaCl; 4) a fraction associated with the nuclear matrix, as described previously.

The relative DNA content in the fractions was determined by measuring the concentration of ^{14}C-thymidine, and the absolute content was determined by Barton's method or by measuring optical density, assuming that a concentration of 1 mg/ml gives absorption of 20 units at 260 nm.

EXPERIMENTAL RESULTS

The level of repair of benz(a)pyrene-damaged DNA can be monitored by determining removal of the carcinogen, covalently bound with DNA. For instance, if embryonic hamster cells were incubated for 24 h with ^{3}H-benz(a)pyrene, and the medium was then replaced by fresh without benz(a)pyrene or serum, the ratio between the nuclear fractions according to the level of carcinogen bound with DNA was not significantly changed after 1-3 days (Fig. 1).

Removal of benz(a)pyrene metabolites bound with DNA thus takes place at about the same rate in all nuclear fractions: the initial ratio between them was preserved. These results mean that approximately the same portion of DNA is repaired in the same time in each nuclear fraction.

Another approach to monitoring the level of repair is to measure the quantity of ^{3}H-thymidine incorporated into DNA. If this approach revealed the character of dependence of thymidine incorporation on time, similar to that examined in Fig. 1 (the ratio between the fractions would remain the same in time), this would mean that there is no need for this DNA to switch from one fraction to another for DNA damaged by the carcinogen to undergo repair.

If, however, after addition of ^{3}H-thymidine to the cells its level was higher in any particular fraction than in another, but it decreased in time and approximated to the radioactivity of total DNA, whereas in the other fraction, conversely, the ^{3}H-thymidine level rose from 0 to the total DNA level, this would mean that for repair to take place, the DNA region with the adduct would have to move from the second fraction into the first, and when repair was complete, it would have to move back again. The experimental curves shown in Fig. 2 are in fact of this type.

Immediately after addition of ^{3}H-thymidine to the confluent culture of embryonic hamster cells, incubated with benz(a)pyrene, the initial level of incorporation of the label into the

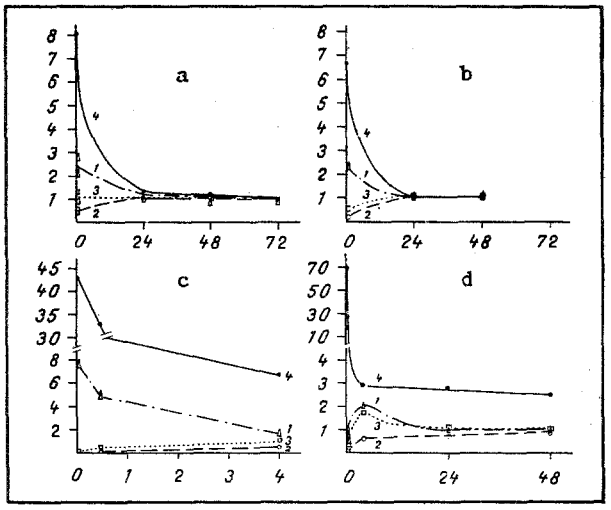

Fig. 2. Dependence of relative concentration of ^{3}H-thymidine in DNA of nuclear fractions on time. Abscissa, time after addition of ^{3}H-thymidine to cells (in h); ordinate, ratio of ^{3}H-thymidine in the fraction indicated to ^{3}H-thymidine concentration in total nuclear DNA. a, b, c) Nuclei fractionated with restriction endonuclease HaeIII; d) nuclei fractionated with micrococcal nuclease; a, c, d) DNA not isolated from nuclear fractions; b) DNA isolated by the phenolic method from fractions indicated in a; c) hydroxyurea added 2 h before ^{3}H-thymidine; d) hydroxyurea added 19 h before ^{3}H-thymidine.

nuclear matrix fraction was restored (Fig. 2a), whereas in the fraction extracted from the nucleus in buffer with low ionic strength, the initial incorporation of the label was close to zero. However, after 24 h the level of the label in these fractions approximated to the level in total DNA. Two other fractions occupied an intermediate position as regards the level of thymidine incorporation between the nuclear matrix fraction and the fraction extracted in buffer with low ionic strength. These ratios between the fractions were preserved in the DNA isolated from these fractions also (Fib. 2b).

In the experiments whose results are given in Fig. 2a, b, three factors contributed to the inhibition of replicative DNA synthesis: confluence of the cell culture, the absence of serum in the medium, and the inhibitory action of benz(a)pyrene metabolites on replication [5].

The results of experiments in which hydroxyurea also was used to inhibit replicative synthesis are shown in Fig. 2c, d. Hydroxyurea reduces the production of deoxyribonucleotide precursors of DNA synthesis, and thus inhibits replication [2, 9]. DNA repair taking place under these conditions is delayed [3, 9].

Under these conditions also the curves reflecting ^{3}H-thymidine incorporation into DNA of the nuclear matrix fractions and the fraction extracted from the nucleus in buffer with low ionic strength, were opposite in character.

The possibility of association of the repair of UV-damaged DNA with the nuclear matrix was examined in [6, 7]. It was postulated in [7] that the matrix is not involved in the process of reparative synthesis, on the basis of a uniform distribution of ^{3}H-thymidine inside the DNA loops. However, this result could be explained on the grounds that during incubation with ^{3}H-thymidine the number of DNA segments completely repaired and detached from the matrix was significantly greater than the number of segments repaired at the same time and attached to the matrix.

The authors in [6] showed that most of the DNA regions undergoing repair in the spread DNA of the nucleotide lie above the matrix. These authors concluded that injuries to DNA induced by UV light are repaired on the nuclear matrix.

The preservation of roughly the same ratio between the fractions in the level of unrepaired benz(a)pyrene adducts (Fig. 1) and the initial incorporation of ^{3}H-thymidine into DNA close to the nuclear matrix, independently of the time of its addition to the cell medium with

hydroxyurea (Fig. 2), and the gradual equalization of the ^{3}H-thymidine concentration in the fractions are all evidence that repair of DNA injuries produced by benz(a)pyrene takes place on the nuclear matrix.

The results of this investigation thus suggest that the nuclear DNA fragment belonging to a particular fraction attaches itself for repair to the nuclear matrix, after which it is returned to those DNA regions that correspond to the initial fraction.

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REACTION OF MONOCLONAL A6/1 ANTIBODIES WITH THE DETERMINANT ANTIGEN OF TUMOR CELL ULTRASTRUCTURES OF EPITHELIAL TISSUES OF EPIDERMAL TYPE

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The nature of differential tissue-specific antigens characteristic of cells of the cambial layers of epithelial tissues of epidermal type, including those characteristic of cells of the basal layer of various types of stratified epithelium, has been studied during recent years [2, 3, 7, 8]. It has been shown that most tissue-specific antigens of epithelial tissues belong to the class of cytoskeletal cell proteins (keratins [5, 6, 8]). We know that the least differentiated (cambial) cells of epithelial tissues of epidermal type have proteins (prekeratins) that are characteristic only of that particular stage of maturation of cells belonging to tissues of that type [2, 7, 8]. It is these compounds which are preserved in the cells of tumors histogenetically connected with epithelial tissues of epidermal type, during tumor transformation [2, 4, 7].

The aim of this investigation was to study the reaction of monoclonal A6/1 antibodies with cell ultrastructures of certain tumors.

An indirect method was adopted, using peroxidase-labeled antibodies. Monoclonal antibodies to antigen of cells from the basal layer of various types of stratified epithelium of the A6/1 series were obtained by the method described previously [1]. The frozen sections were processed and ultrathin sections cut by the usual method. Tissues of squamous-cell carcinoma from various locations (larynx, esophagus, cervix uteri, lung) were investigated. The results showed that A6/1 antibodies reacting with the antigen characteristic of cells from the basal layer of stratified epithelia and cells of other epithelial tissues of epidermal

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