ENERGY DEPENDENT NUCLEOLYTIC PROCESSES ARE RESPONSIBLE FOR THE PRODUCTION OF MANY POST-IRRADIATION BREAKS IN L CELL DNA

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SUMMARY

L cells irradiated while in growth medium or in a balanced salts solution showed a rapid appearance of DNA breaks characterized by 5' phosphoryl termini. Following irradiation, the number of breaks quickly reached a maximum, after which they were rapidly repaired. Dinitrophenol, an inhibitor of aerobic oxidative phosphorylation, prevented the DNA breaks from appearing.

INTRODUCTION

Recently, we postulated that the "cut-and-patch" mechanism plays a major role in the repair of radiation injury by cultured mammalian cells (1). The plausibility of this model rests upon the notion that radiation produces several types of damage to the DNA molecules. Some chemical bonds within the DNA molecule are thought to be broken as the result of the radiation, per se. Other bonds, while not broken are thought to be rendered abnormal and thereby liable to attack by nucleolytic enzymes. According to our concept, these abnormal bonds have a certain potential to return to normal (relax) and no longer be liable to the attack of these nucleolytic enzymes. The ultimate fate of a given bond abnormality, then, would seem to be a consequence of the competition between the nucleolytic enzymes and the tendency of the abnormalities to "relax."

While the action of nucleolytic enzymes in the repair of radiation injury has been demonstrated for bacteria, the evidence for mammalian cells is largely

speculative. Experiments have shown that bacteria extensively degrade DNA after irradiation (2). For cultured mammalian cells, on the other hand, large doses of radiation do not result in this extensive DNA degradation (3).

For bacterial systems the degradation seems to be energy dependent. The inhibitor of aerobic oxidative phosphorylation, 2,4-dinitrophenol (DNP), prevents the post-irradiation DNA degradation (4). Unfortunately, the energy dependence (or even the existence) of nucleolytic enzymes in the repair of radiation damage by mammalian cells cannot be measured by methods based upon DNA degradation.

In an earlier paper we described the measurement of radiation-produced DNA breaks by polynucleotide kinase (5). This method allows the detection of those breaks which are characterized by 5' phosphoryl termini. Under the conditions of the assay, the "cold" 5' phosphoryl termini are replaced with $^{32}PO_4$. Our results with mouse liver and L cell DNA showed these breaks to be rapidly repaired after irradiation.

For the present investigation we measured the number of radiation induced DNA breaks in L cells which were treated with DNP. These results were contrasted with data from cells which were kept in a balanced salts solution.

METHODS

The techniques used for culturing the cells, the irradiations, and the polynucleotide kinase assay have previously been described (5-7). For the present studies the cells were carried as monolayers prior to their use. The cells were detached from the monolayer bottles with dilute trypsin, pooled, washed twice with glucose-free Hank's balanced salt solution (HBSS), and divided into two portions. The cells of the first portion were suspended in 1×10^{-4}M DNP (dissolved in HBSS), while the cells of the second portion were suspended in HBSS (without DNP). The cells were suspended at a concentration of $3\text{-}5 \times 10^6$ cells per ml. Both suspensions were gently agitated with a magnetic stirrer. The suspensions were kept at room temperature (22°C).

Immediately before irradiation (1000 rads; 400 rads/min; 250 kVp x-rays), 3 ml samples were taken from both suspensions. The samples were delivered into

screw-capped test tubes which were simultaneously irradiated. At appropriate times after exposure, the tubes were plunged into dry ice--acetone and allowed to cool until the fluid was almost solid. The tubes were then rapidly centrifuged at 4°C and the cell pellet processed as previously described (5). The sham irradiated controls (HBSS and DNP) were handled as pairs, as were the irradiated cells.

RESULTS AND DISCUSSION

Fig. 1 shows the results of a typical experiment. Notice the contraction of the abscissa time scale after 5 minutes. The DNA specific activity (cpm/μg) is proportional to the number of 5' phosphoryl termini present in the sample. During the first 0.5 minutes after irradiation, the number of measurable breaks increased rapidly. After 0.5 minutes, however, the number of breaks decreased

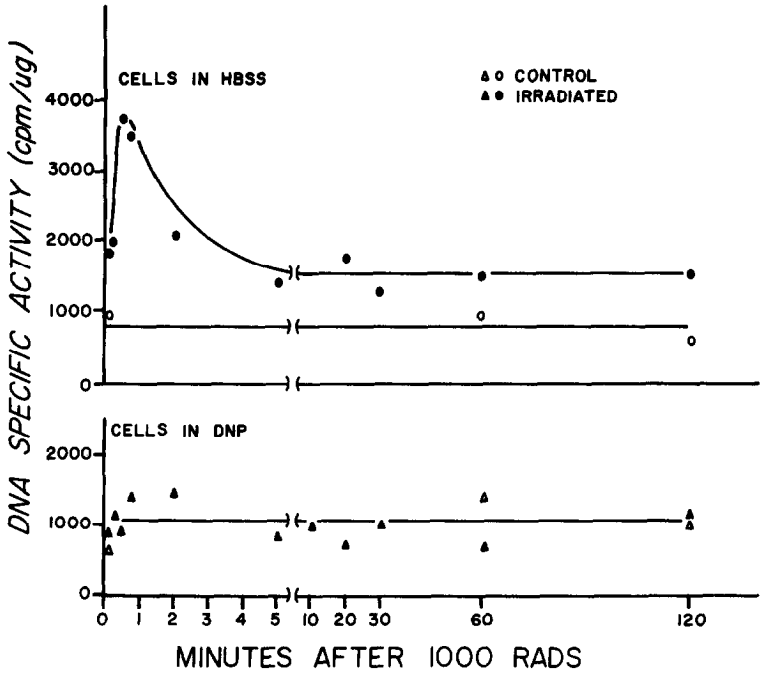

Fig. 1. The upper portion shows the results for cells suspended in HBSS (circles), while the lower portion shows the results for cells suspended in 1 x 10^{-4}M DNP (triangles). The open circles and triangles are the controls. The abscissa scale has been contracted after 5 minutes. Notice that while a definite pattern of increase followed by a decrease of the DNA specific activity (a measure of 5' phosphoryl termini) characterized the cells in HBSS, DNP obliterated this pattern.

in approximately an exponential manner. The pattern of this repair is similar to that reported earlier for cells in complete growth medium (5).

For cells in DNP, however, the sharp increase in measurable 5' phosphoryl termini did not appear. Instead, the number of measurable 5' phosphoryl termini for the DNP treated cells remained near control values. In earlier studies, we showed that although DNP prevented ATP and macromolecular synthesis, the effects were easily reversed by replacing the DNP with growth medium (1, 7). In fact, periods of contact to DNP up to 24 hours causes no loss of viability.

We have irradiated aqueous solutions of DNA (salmon sperm) in vitro. Very few, if any, 5' phosphoryl termini, above control, were found for doses of the order of 1000 rads. The intervention of endogenous processes, then, seem to be required for the production (and presumably the repair) of most of the DNA breaks.

In our earlier studies we did not detect the rapid increase in breaks which occurred during the first 30 seconds after irradiation (5). Since the first point measured in the earlier study was 30 seconds after the dose, the "rising" component was not found. The collection of samples very soon after irradiation, then, was necessary to demonstrate this structure.

Kornberg, in a recent review article summarized the properties of DNA polymerase (8). While one usually thinks of this enzyme as directing the synthesis of DNA, it also has nucleolytic properties. Perhaps the nucleolytic aspect of DNA polymerase could be responsible for the conversion of radiation induced DNA abnormalities into actual breaks. Since other nucleolytic enzymes are present within the cell, certainly they could be responsible for producing the breaks instead of DNA polymerase. As more data become available, perhaps these questions will be answered.

In an earlier paper we indicated a reservation which certainly remains valid for the present work (5). Very possibly DNA breaks characterized by 5' phosphoryl termini do not represent the only type of break caused by radiation. Other types of breaks may be produced and they may or may not be repaired by similar processes. Hopefully, experiments from ours and other laboratories will

soon answer this question.

In conclusion, we believe that our results (present and earlier) indicate that 1) nucleolytic enzyme activity markedly increases the number of DNA breaks which appear after irradiation, 2) the enzyme activity is energy dependent, and 3) the nucleolytic activity does not involve extensive DNA degradation. The results suggest that, indeed, a "cut-and-patch" mechanism plays a key role in the repair of radiation damaged DNA.

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