*Proc. Natl. Acad. Sci. USA*
Vol. 77, No. 12, pp. 7315–7317, December 1980
Cell Biology

# Radiosensitivity in ataxia-telangiectasia: A new explanation

(genetic disease/cell-cycle delays/replicon initiation/mitotic delay/chromosome aberrations)

R. B. PAINTER AND B. R. YOUNG

Laboratory of Radiobiology, University of California, San Francisco, California 94143

*Communicated by J. Herbert Taylor, September 8, 1980*

**ABSTRACT** **The cause of increased radiosensitivity in ataxia-telangiectasia (AT) cells may be a defect in their ability to respond to DNA damage rather than a defect in their ability to repair it. Doses of x-radiation that markedly inhibited the rate of DNA synthesis in normal human cells caused almost no inhibition in AT cells and thus less delay during which x-ray damage could be repaired. The radioresistance of DNA synthesis in AT cells was primarily due to a much smaller inhibition of replicon initiation than in normal cells; the AT cells were also more resistant to damage that inhibited chain elongation. AT cells have been reported to undergo less radiation-induced mitotic delay than normal cells, which may cause them to move from $G_2$ phase into mitosis before repair is complete and may result in the increased incidence of chromatid aberrations observed by others. Therefore, AT cells fail to go through those delays that allow normal cells to repair DNA damage before it can be expressed.**

The genetic disease ataxia-telangiectasia (AT) has many pathologic features. One of these is a greatly increased sensitivity to ionizing radiation (1), but the others are not dependent on overt exposure to a DNA-damaging agent. In contrast, the genetic disease xeroderma pigmentosum, which is defective in DNA repair, is expressed in most patients only in response to the DNA-damaging agent ultraviolet light. Paterson *et al.* (2) reported that 7 of 12 AT cell strains showed a defect in repair replication after high doses of x-radiation administered in anoxia. However, those AT cells that were proficient in repair replication were as sensitive to x-radiation as those that were defective, so there is no correlation in this disease between the excision repair defect and the cells' sensitivity to the DNA-damaging agent. Thus, it is very possible that in AT, unlike xeroderma pigmentosum, the repair defect is a consequence and not a cause of the principal cellular abnormality. We report here a response to ionizing radiation by AT cells that with other known features of these cells can explain their radiosensitivity and, perhaps, other clinical manifestations of this disease.

## MATERIALS AND METHODS

**Cell Lines.** Cell strains AT3BI and AT5BI were generously supplied by D. G. Harnden and A. M. R. Taylor, University of Birmingham, England. The AT3BI strain has been classified as defective and the AT5BI as proficient in repair replication after x-irradiation (2). Normal human diploid cell strain E-11 was the gift of A. J. Millis, State University of New York, Albany, NY. All cells were grown in Eagle's minimal essential medium supplemented with 15% (vol/vol) fetal bovine serum.

**Measurement of Inhibition of DNA Synthesis by X-Radiation.** The AT and normal cells, at passages between 10 and 15, were inoculated into 35-mm petri dishes at about $5 \times 10^4$ cells per dish. After 1 day of growth, the medium was replaced by one containing 0.01 $\mu$Ci (1 Ci = $3.7 \times 10^{10}$ becquerels) of [$^{14}$C]thymidine (specific activity, 50 mCi/mmol) per ml, and incubation was continued for 24 hr. The medium was then removed, and two dishes containing AT cells and two containing E-11 cells were simultaneously exposed to 0–5000 rad of x-rays (300 kVp max, about 1200 rad/min). Immediately after irradiation, nonradioactive medium was placed in all dishes and the cells were returned to the incubator for 30 min. This medium was then removed, and all cultures were incubated for 10 min with medium containing 20 $\mu$Ci/ml of [$^3$H]thymidine (50 Ci/mmol). This medium was then rapidly removed, and the cells were washed with ice-cold 0.15 M sodium chloride/0.015 M sodium citrate and scraped into the saline citrate solution; ice-cold 4% perchloric acid was added, and the cell suspension was filtered through GF/C (Whatman) filters, which were rinsed sequentially with 4% perchloric acid, 70% (vol/vol) alcohol, and 100% alcohol, dried, and assayed for radioactivity in a liquid scintillation spectrometer. The resulting $^3$H/$^{14}$C ratios were measures of the specific activity of DNA and, therefore, of the rate of DNA synthesis.

**Alkaline Sucrose Gradient Analysis.** Cells were irradiated, incubated for 30 min, pulse-labeled with [$^3$H]thymidine for 10 min, and lysed on top of alkaline sucrose gradients; the DNA was then centrifuged through the gradients, which were fractionated and analyzed for radioactivity (3).

## RESULTS

The dose–response curves for inhibition of DNA synthesis in E-11 normal diploid cells by x-radiation (Fig. 1) showed a steep decline at low doses and a shallow decline at high doses, as has been reported for mammalian cells many times (for reviews, see refs. 4 and 5). The dose–response curve for AT3BI cells, however, showed no steep component; it was almost identical to the shallow component of the curve for normal cells. For AT5BI cells, very similar results were obtained (data not shown). It has been established that the steep component of DNA synthesis inhibition by x-radiation is primarily caused by inhibition of replicon initiation (6–8). The shallow component is believed to be caused by blocked chain elongation in individual replicons (9). Therefore, our interpretation of these data is that the DNA damage that inhibits replicon initiation in normal human cells fails to do so in AT cells.

To confirm this interpretation, alkaline sucrose gradient analyses were performed. The major difference between profiles of irradiated AT3BI and E-11 cells (Fig. 2) was that for E-11 cells there was a large reduction of radioactivity at low molecular weight regions (fractions 4–10) compared to unirradiated cells, whereas for AT cells there was only a small reduction of radioactivity at low molecular weight regions compared to unirradiated cells. The results with AT5BI cells also showed that the incorporation into low molecular weight DNA was inhibited much less than in E-11 cells (data not

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: AT, ataxia-telangiectasia.

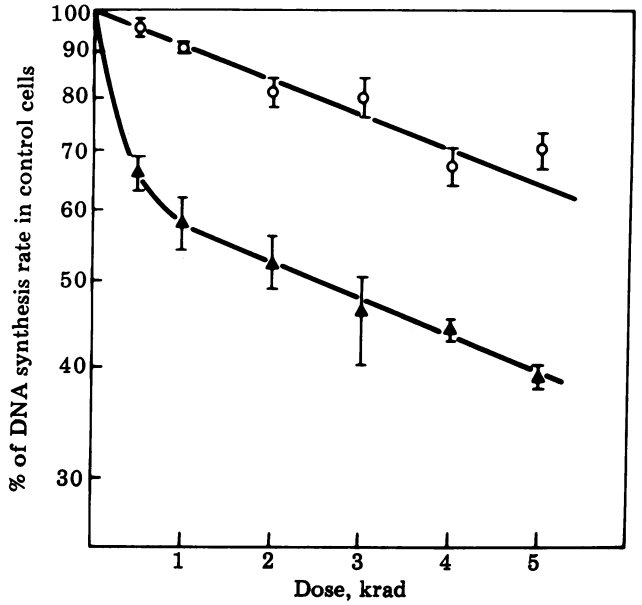


FIG. 1. Inhibition of DNA synthesis rate as a function of x-radiation dose in E-11 normal diploid cells (▲) and in AT3BI AT cells (○). Error bars indicate ranges of duplicate cultures.

shown). These results show that replicon initiation was inhibited by x-radiation to a much lesser extent in AT cells than in E-11 cells.

Somewhat surprisingly, these profiles indicate that chain elongation was also more resistant to x-radiation in AT cells than in E-11 cells. For AT cells, the higher molecular weight peaks and descending limbs of the profiles (fractions 13–24) from irradiated and unirradiated cells were almost superimposable, whereas for E-11 cells, the higher molecular weight peaks and descending limbs of the profiles of irradiated cells showed a dose-dependent departure downward from corresponding regions in the profile of unirradiated cells. These regions of the profiles reflect chain elongation and joining of nascent DNA (3). Thus, although a block to initiation is the component of DNA synthesis that was inhibited to the greatest extent by x-radiation in normal human cells, chain elongation processes were slightly inhibited in normal human cells but not in AT cells.

## DISCUSSION

Our results indicate that AT cells fail to respond to the x-ray-induced DNA damage that in normal cells inhibits initiation of replicons and blocks chain elongation. The exact reason for this is unclear. One possibility is that AT cells lack a factor or process that in normal cells delays replication after irradiation. If such a factor exists, however, it can only interact specifically with those parts of the DNA that contain damage. Povirk (10) showed that DNA synthesis was inhibited only in the damaged bromodeoxyuridine-substituted regions of DNA after cells were irradiated with 313-nm light, whereas regions of DNA not substituted with bromodeoxyuridine (and therefore resistant to 313-nm light) replicated at normal rates.

Another possibility, which we favor, is that the DNA damage induces a conformational change in replicon clusters that makes it impossible for synthesis to begin in any of the replicons within those clusters until damage is repaired (4, 11). We have conjectured (12) that the replicon cluster is identical to the structural subunit of chromatin originally identified by Cook and Brazell (13), because both have target molecular weights of about $10^9$ (6, 13, 14) and because the same damage that changes

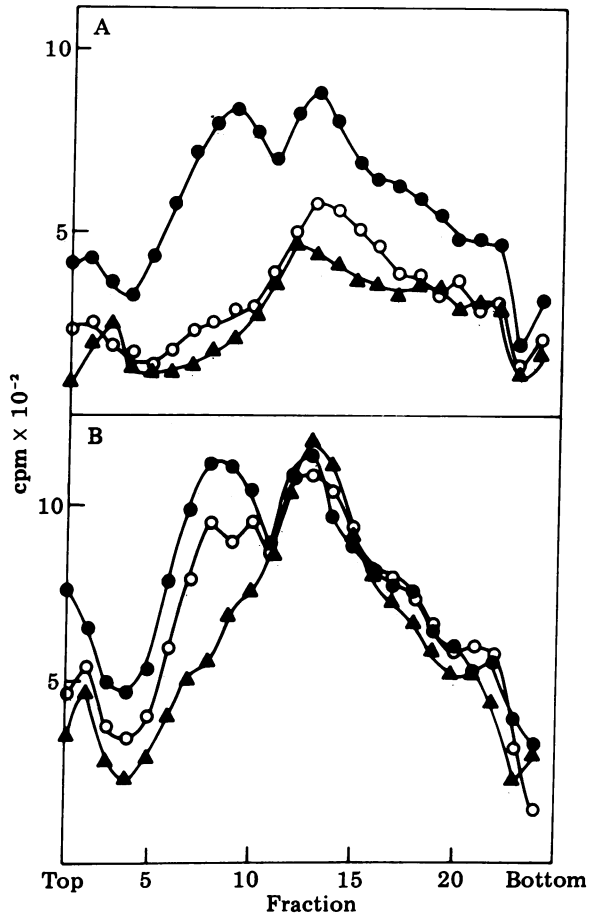


FIG. 2. Alkaline sucrose gradient profiles of the DNA (labeled in a 10-min pulse) of E-11 normal diploid cells (*A*) and AT cells (*B*) 30 min after irradiation with 2000 (▲), 500 (○), or 0 (●) rad of x-rays. Relative rates of DNA synthesis for normal diploids were 52% of control after 500 rad and 40% of control after 2000 rad; for AT cells, the relative rates of DNA synthesis were 94% of control after 500 rad and 80% of control after 2000 rad.

the conformation of the structural subunits *in vitro* also blocks replicon initiation. According to this hypothesis, inhibition of replicon initiation is a passive process. If this is so, AT cells must have an altered chromatin structure that does not respond to x-ray-induced damage. Such a change in chromatin could have severe consequences for the cell and might be the basis for other features of the disease.

Whatever the reason for their radioresistant DNA synthesis, the failure of AT cells to respond to chromatin damage may also explain other results with AT cells. Zampetti-Bosseler and Scott (15) found that the mitotic delay induced by 150 rad in AT cells (about 4 hr) was much less than that induced in normal diploid human cells (about 7 hr). Failure of AT cells to delay in $G_2$ phase may cause them to progress into mitosis before repair is complete and could be the basis for the increased frequency of chromatid aberrations that has been observed in AT cells (16).

The responses of AT cells to ionizing radiation are very similar to those observed when other mammalian cells are irradiated in the presence of caffeine, which (*i*) prevents inhibition of DNA synthesis (17) by reversing the block to replicon initiation (18), (*ii*) shortens mitotic delay (19), (*iii*) increases the incidence of chromosomal aberrations (20), and (*iv*) enhances cell killing (21, 22). We suggest, therefore, that AT cells are like caffeine-treated normal cells in that their radiosensitivity is not caused by their inability to repair damage but by their failure

to go through those x-ray-induced delays that allow normal cells to repair damage before it can be expressed.

The results discussed here imply that sensitivity to a DNA-damaging agent can be due to defects in systems other than those that participate directly in the modification of DNA lesions.

We thank Ms. Mary McKenney for valuable suggestions in the preparation of this paper. This work was supported by the U.S. Department of Energy.

1. Taylor, A. M. R., Harnden, D. G., Arlett, C. F., Harcourt, S. A., Lehmann, A. R., Stevens, S. & Bridges, B. A. (1975) *Nature (London)* **258,** 427–429.
2. Paterson, M. C., Smith, B. P., Knight, P. A. & Anderson, A. K. (1977) in *Research in Photobiology,* ed. Castellani, A. (Plenum, New York), pp. 207–218.
3. Painter, R. B. & Young, B. R. (1976) *Biochim. Biophys. Acta* **418,** 146–153.
4. Okada, S. (1970) *Radiation Biochemistry* (Academic, New York), Vol. 1.
5. Walters, R. A. & Enger, M. D. (1976) *Adv. Radiat. Biol.* **6,** 1–48.
6. Makino, F. & Okada, S. (1975) *Radiat. Res.* **62,** 37–51.
7. Walters, R. A. & Hildebrand, C. E. (1975) *Biochem. Biophys. Res. Commun.* **65,** 265–271.
8. Painter, R. B. & Young, B. R. (1975) *Radiat. Res.* **64,** 648–656.
9. Watanabe, I. (1974) *Radiat. Res.* **58,** 541–556.
10. Povirk, L. F. (1977) *J. Mol. Biol.* **114,** 141–151.
11. Povirk, L. F. & Painter, R. B. (1976) *Biochim. Biophys. Acta* **432,** 267–272.
12. Painter, R. B. (1978) *Cancer Res.* **38,** 4445–4449.
13. Cook, P. R. & Brazell, I. A. (1975) *J. Cell Sci.* **19,** 261–279.
14. Painter, R. B. & Rasmussen, R. E. (1964) *Nature (London)* **201,** 162–165.
15. Zampetti-Bosseler, F. & Scott, D. (1980) *Intl. J. Radiat. Biol.*, in press.
16. Taylor, A. M. R. (1978) *Mutat. Res.* **50,** 407–418.
17. Tolmach, L. J., Jones, R. W. & Busse, P. M. (1977) *Radiat. Res.* **71,** 653–665.
18. Painter, R. B. (1980) *J. Mol. Biol.*, in press.
19. Walters, R. A., Gurley, L. R. & Tobey, R. A. (1974) *Biophys. J.* **14,** 99–118.
20. Kato, H. (1973) *Exp. Cell Res.* **82,** 383–390.
21. Nilsson, K. & Lehmann, A. R. (1975) *Mutat. Res.* **30,** 255–266.
22. Busse, P. M., Bose, S. K., Jones, R. W. & Tolmach, L. J. (1977) *Radiat. Res.* **71,** 666–677.