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Repair of Radiation and Chemical Damage to DNA in Human Cells

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104

Running head: Repair of Human DNA

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DNA is susceptible to damage from extracellular sources. Repair of damaged DNA can be divided into (a) prereplication repair, observed in DNA that is present when the insult is given, and (b) postreplication repair, observed in the daughter strands synthesized after the insult. Recovery and repair in human cells have been measured in a number of ways: (a) by a decrease in reproductive death with dose fractionation (1), (b) by unscheduled DNA synthesis (2), (c) by repair replication (3), (d) by sedimentation-velocity studies (4), (e) by a combination of repair replication and sedimentation-velocity studies with photolysis of BrdUrd-sensitized DNA (5), and (f) by excision of chemical products or photoproducts from DNA (6,7).

Recovery of human cells from radiation damage was first described by Lockart et al. (1), who noted that fractionated doses resulted in a lower level of reproductive death than did the same amounts of radiation given in single doses. Possible repair of radiation damage to DNA was first detected when unscheduled DNA synthesis was observed after ultraviolet (2) and ionizing (3) irradiation. The types of damage and modes of repair were soon examined and are described below.

PREREPLICATION REPAIR

Repair of Damage from Ultraviolet Irradiation

Repair of ultraviolet (uv) damage involves four steps: (a) incision of the DNA near the dimer, (b) DNA degradation, (c) DNA synthesis (repair replication), and (d) joining of the newly synthesized DNA to the old strand (ligase action).

The presumed repair of DNA after uv irradiation of human cells was first detected by Rasmussen and Painter (2), using autoradiography to detect unscheduled (non-S-phase) DNA synthesis after irradiation. The same authors (3) confirmed their results by showing that after uv irradiation exogenous nucleotides are inserted into the parental DNA (repair replication). Regan et al. (7) demonstrated the presence of pyrimidine dimers in uv-irradiated human DNA and their subsequent removal during postirradiation incubation. Cleaver (8) found that in cells derived from individuals with xeroderma pigmentosum (XP) repair replication was reduced or absent. Such individuals are sensitive to sunlight, as indicated by the appearance of skin cancer at an early age. Studies on cells from these individuals were expanded by Setlow et al. (9), who showed that XP cells do not excise pyrimidine dimers and do not make the initial incision in the DNA containing the dimer.

The kinetics and mechanisms of repair were examined in more detail by Evans and Norman (10), who studied unscheduled DNA synthesis, and by Regan and Setlow (11), who studied the sedimentation velocities of photolyzed, BrdUrd-containing DNA from uv-irradiated human cells. After uv irradiation, unscheduled DNA synthesis is essentially complete after 3–4 hr (Fig. 1). The size and number of repaired

regions were investigated by Regan et al. (5), using the photolysis technique, by which the length and number of repaired regions can be determined. They found 0.33–0.50 repaired regions per pyrimidine dimer and ~100 replaced nucleotides per repaired region. The pattern of photolysis of repaired uv damage was curvilinear (Fig. 2), indicating that saturation had been achieved at higher doses of 313-nm radiation, as expected.

F-2

Repair of Damage from Ionizing Radiation

Possible repair of damage from ionizing radiation was first observed as unscheduled DNA synthesis by Rasmussen and Painter (3). The production of breaks in human DNA by ionizing radiation and their subsequent rejoining (repair) was first reported by Lohman (4), using sedimentation-velocity analysis of DNA from X-irradiated human cells. And recently, Zhestyanikov et al. (12) described a human mutation that causes a lack of repair of damage from ionizing radiation. The kinetics of unscheduled DNA synthesis were studied in detail by Spiegler and Norman (13), who observed that this repair system could be divided into a rapid component, which operates within the first hour after irradiation, and a slower component, which continues through 22 hr (Fig. 3). Regan and Setlow (11), using the technique of photolysis after repair replication, studied the mechanics and kinetics of repair and photolysis of γ -irradiated human DNA and found that three or four nucleotides are replaced per repaired region. Figure 4 shows the kinetics of photolysis of the repair-replicated regions. The curve is a slowly rising linear

F-3

F-4

line and can be contrasted with the curvilinear line obtained in studies of the repair of uv damage (Fig. 2). The data from studies of unscheduled synthesis after ionizing and uv irradiation (Figs. 1 and 3) should also be contrasted.

To summarize the above observations, there are at least two prereplication repair systems: one (uv-type repair) is exemplified by the repair of damage from uv radiation, operates slowly (up to 4 hr), and replaces large amounts of DNA (~100 nucleotides per repaired region); the other (ionizing-type repair) is exemplified by repair of damage from ionizing radiation and consists of two components—rapid repair, which is essentially complete in 1 hr, and a slower repair, which continues through 22 hr after irradiation—both of which replace three or four nucleotides per initial chain break.

Repair of Chemical Damage

The ability of human cells to recover from chemical damage was first described by Crathorn and Roberts (6), who showed chromatographically that HeLa cells excise the chemical products of ^{35}S -labeled mustard gas from DNA and have survival curves that indicate the presence of repair systems. Workers in the same laboratory (15, 16) expanded on these results by demonstrating the presence of repair replication and the recovery of the ability to synthesize DNA at the normal rate after treatment with mustard gas.

Chemicals that damage DNA can be divided into three classes, based on the kinetics and mode of the repair system that acts on their damage. Table 1 is a compilation of chemicals that have been examined and the types of repair system

they evoke. There are three types of compounds: those which evoke the rapid (ionizing-type) repair system, with the insertion of only a few nucleotides per repaired region; those which evoke the slower (uv-type) repair system, with the insertion of 80–100 nucleotides per repaired region; and those which evoke a combination of the two repair systems. From the data in Table 1 it is apparent that the enzyme that is defective in XP is not specific for uv damage but must recognize damage from a broad spectrum of insults. The common parameter of all the agents not repaired by XP is that they produce only a few single-strand breaks but distort the DNA structure. One can hypothesize that the human mutant lacking repair of ionizing radiation damage (12) would also be deficient in repair of damage from compounds that evoke the ionizing-type repair system.

POSTREPLICATION REPAIR

Ultraviolet Radiation

DNA synthesized by human cells after uv irradiation is synthesized in smaller ($8-10 \times 10^6$ daltons) than normal segments ($\sim 40 \times 10^6$ daltons), as assayed on alkaline sucrose gradients after pulse-labeling with [^3H]thymidine (Fig. 5a,b). When uv-irradiated, pulse-labeled cells are incubated, the short segments are elongated to form high-molecular-weight ($> 10^8$ daltons) DNA (Fig. 5c,d). Table 2 shows that the size of the DNA segments synthesized after a given dose of uv radiation is comparable to the average distance between pyrimidine dimers produced at the same dose (20,21). As a further investigation of the effect of

(F-5)

(T-2)

dimers on the production of short segments of DNA after uv irradiation, the following experiment was done. Normal (excision-plus) and XP (excision-minus) human cells were incubated in medium containing hydroxyurea for 2 hr to stop normal DNA synthesis, after which they were uv-irradiated and incubated in hydroxyurea for various times. The medium was then changed before pulse-labeling and sedimentation. Figure 6 shows results from these experiments. It is apparent that human cells slowly recover the ability to synthesize DNA in segments of normal size and that the presence or absence of dimers does not affect the size of the DNA units synthesized long after irradiation.

(F-6)

We also examined the elongation and joining process that operates on the shorter DNA segments synthesized after uv irradiation and on the intermediate segments synthesized by untreated cells (22). Using the photolysis technique (Fig. 7), we showed that the intermediate segments synthesized by untreated cells are elongated by insertion of large amounts of exogenous nucleotides (normal semiconservative DNA replication) and that the shorter segments synthesized after uv irradiation are elongated and joined by insertion of small amounts ($\sim 10^3$ nucleotides, which are photolyzed at higher 313-nm doses) in addition to the large amounts of exogenous nucleotides (photolyzed at lower 313-nm doses) observed in the untreated cells. Figure 8 shows a model of DNA chain elongation and joining of the short segments synthesized in human cells shortly after uv irradiation.

(F-7)

(F-8)

Ionizing Radiation

Our results indicate that irradiation with 10 krad from a ^{60}Co source under oxic conditions does not reduce the size of the DNA segments synthesized by human cells during the first 4 hr after irradiation (S. N. Buhl and J. D. Regan, unpublished results).

N-acetoxy-2-acetylaminofluorene

Treatment with either 70 or 7 μM N-acetoxy-2-acetylaminofluorene for 1 hr does not affect the size of the DNA segments synthesized in human cells during the first 6 hr after treatment, nor is the process of DNA chain elongation and joining severely affected (S. N. Buhl and J. D. Regan, unpublished results). The damage induced by this compound is repaired by uv-type prereplication repair (Table 1).

Methyl Methanesulfonate

Treatment of human cells with methyl methanesulfonate (MMS) reduces the rate of DNA synthesis for several hours; however, the amount of DNA synthesis returns to normal within 4 hr after a 1-hr treatment (Fig. 9). [A similar observation was made in HeLa cells treated with mustard gas (16).] MMS also has a differential effect on the size of the DNA segments synthesized. Figure 10 shows the differences between the reciprocals of the weight-average molecular weights of DNA from untreated and treated cells pulse-labeled at various times after a 1-hr treatment with MMS. The important observation is the reduction in the size of the units

(F-9)

(F-10)

synthesized 2.5 hr after treatment and the recovery of the ability to synthesize DNA in normal lengths within 4 hr after treatment (23). As a test of whether the cells could elongate and join the DNA after MMS treatment, they were incubated for 12 hr after pulse-labeling at various times after MMS treatment. Figure 11 shows that the shorter segments ($\sim 20 \times 10^6$ daltons) synthesized at 2.5 hr are elongated and joined to form high-molecular-weight DNA ($> 10^8$ daltons), as are the intermediate segments ($> 35-40 \times 10^6$ daltons) synthesized by nontreated cells and by treated cells pulse-labeled at 0.5 and 4.0 hr after MMS treatment.

(F-11)

To summarize the postreplication repair systems briefly, there are two general types, one exemplified by the elongation and joining of the short segments synthesized after the insult and the other by the recovery of the ability to synthesize DNA in segments of normal size rather than continuing to synthesize the shorter segments observed immediately after the insult. Recovery of the ability to synthesize DNA in normal segments is faster after MMS treatment than after uv irradiation. Perhaps this difference represents a difference in repair systems. Similarly, the process of chain elongation and joining after MMS treatment needs to be examined to determine whether it differs from the elongation and joining process observed after uv irradiation.

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SUMMARY

There is little correlation of the observations made in studies of prereplication and postreplication repair. In the case of damage from uv radiation, the size of the DNA segments synthesized shortly after uv irradiation is equal to the distance between pyrimidine dimers. However, this correlation does not hold for DNA synthesized at long times after uv irradiation. The data summarized here indicate that the dimer is not the lesion that determines the size of the DNA segments synthesized on uv-irradiated templates, but these data are not conclusive. Another example of the lack of correlation between results from studies of prereplication and postreplication repair is the data from cells incubated in N-acetoxy-2-acetylaminofluorene. This agent causes DNA damage that is recognized by the same prereplication repair system that recognizes uv damage, but it has no effect on the size of the DNA segments observed in studies of postreplication repair.

Equally puzzling are the findings for ionizing irradiation and MMS treatment. Although damage from these agents seems to be recognized by the same prereplication repair system, damage from ionizing radiation has no effect on the size of the DNA synthesized after treatment, whereas damage from MMS has a tremendous effect.

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TABLE 1
Repair of Chemical Damage to DNA

Agent	Repair in xeroderma pigmentosum	Type of repair	Reference
<u>N</u> -Acetoxy-2-acetylaminofluorine	no	uv	Setlow and Regan (17)
4-Nitroquinoline oxide	partial	uv and ionizing	Stich and San (18) Regan and Setlow (11)
Ethyl methanesulfonate	not tested	ionizing	Regan and Setlow (11)
Propane sultone	not tested	ionizing	Regan and Setlow (11)
Methyl methanesulfonate	yes	ionizing	Cleaver (19) Clarkson and Evans (14) Regan and Setlow (11)
Nitrogen mustard	reduced	uv	Cleaver (19) Clarkson and Evans (14)
Mitomycin C	not tested	ionizing	Regan and Setlow (11)
ICR-170*	no	uv	Regan and Setlow (11)

* (2-Methoxy-6-chloro-9-[3-(ethyl-2-chloroethyl)-aminopropylaminoacridine dihydrochloride.

TABLE 2
Measured Size of DNA and Distance Between Dimers^{*}

Dose of uv radiation (ergs/mm ²)	Measured dimers [†] (%)	Calculated distance between dimers [†] (10 ⁶ daltons)	Measured size of DNA [§] (10 ⁶ daltons)
200	0.08	2.1	2.1
100	0.04	4.2	3.6-4.5
75	0.03	6.0	4.6-5.2
50	0.02	8.4	6.6-10.3
25	0.01	16.8	15.4

^{*} See refs. 20 and 21.

[†] Measurements provided by W. L. Carrier.

[‡] From Setlow et al. (9).

[§] Number-average molecular weight of the peak, not corrected for the number-average molecular weight resulting from pulse-labeling of unirradiated cells.

FIGURE LEGENDS

Fig. 1. Kinetics of [^3H]thymidine incorporation (unscheduled DNA synthesis) in human lymphocytes incubated at 37°C after uv irradiation with 25 ergs/mm².

The kinetics describe a single-component repair system that completes its repair within 3–4 hr after irradiation. Data obtained from Evans and Norman (10).

Fig. 2. Kinetics of photolysis, indicating repair of DNA in BrdUrd after 200 ergs/mm² of 254-nm radiation. $\Delta(1/\underline{M}_w) = [(1/\underline{M}_w)^{\text{BrdUrd}} - (1/\underline{M}_w)^{\text{dThd}}]$

represents the presence of photosensitive BrdUrd-containing regions in DNA. Normal human cells repair extensively and contain many photosensitive regions, but XP cells repair minimally and contain few photosensitive regions. Data obtained from Regan and Setlow (11).

Fig. 3. Kinetics of [^3H]thymidine incorporation (unscheduled DNA synthesis) into γ -irradiated (5 kR) human lymphocytes. The data describe a two-component repair system—a rapid system, which is complete within the first hour, and a slow system, which continues through 22 hr. Data obtained from Clarkson and Evans (14).

Fig. 4. Kinetics of photolysis of γ -irradiated (10 kR) human DNA repaired in BrdUrd. Increasing $\Delta(1/\underline{M}_w)$ represents the presence of photosensitive BrdUrd-containing regions of DNA. The slowly rising linear line represents small substituted regions with three or four nucleotides per substituted (repaired) region. Data obtained from Regan and Setlow (11).

Fig. 5. Alkaline sucrose gradient sedimentation profiles of DNA from normal human fibroblasts (a) nonirradiated or (b) uv-irradiated with 75 ergs/mm^2 , pulse-labeled with $[^3\text{H}]$ thymidine, and harvested immediately. (c, d) Profiles for (a) and (b), respectively, after incubation for 8 hr. Data obtained from Buhl et al. (20).

Fig. 6. Differences in the reciprocals of M_w for nonirradiated and uv-irradiated human cells pulse-labeled for 30 min at various times after irradiation. Decreasing $\Delta(1/M_w)$ represents recovery of ability to synthesize DNA in normal size segments. Data obtained from Buhl et al. (21).

Fig. 7. The effect of various fluxes of 313-nm radiation on the DNA of nonirradiated and uv-irradiated normal human fibroblasts incubated for 8 hr in 0.1 mM BrdUrd . Cells were irradiated with 0 (O) or $100 \text{ (}\bullet\text{) ergs/mm}^2$, pulse-labeled with $[^3\text{H}]$ thymidine, and incubated in BrdUrd. $1/M_{313}$, reciprocal of the weight-average molecular weight after photolysis with 313 nm and sedimentation; $1/M_0$, reciprocal of the weight-average molecular weight after sedimentation of DNA from cells incubated in BrdUrd. Increasing $\Delta(1/M_w)$ represents additional photosensitive BrdUrd-containing DNA. This additional sensitivity is observed at $0.5 \times 10^5 \text{ ergs/mm}^2$ of 313-nm radiation, which is equivalent to $\sim 10^3$ nucleotides of fully substituted BrdUrd-containing DNA (17). Data obtained from Buhl et al. (22).

Fig. 8. Model describing elongation of DNA chains and joining of the short segments synthesized within the first hour after uv irradiation of human cells.

□, beginning or end of replicating unit; ●, pyrimidine dimer; ■, short segment of DNA observed after pulse-labeling for 30 min; ~~, newly synthesized DNA, elongating and joining of the short segments. The size of the regions was determined by the photolysis technique (22).

Fig. 9. Relative amounts of DNA synthesized by human cells treated with 1 mM MMS for 1 hr at various times after treatment. Data obtained from Buhl and Regan (23).

Fig. 10. Differences between the reciprocals of the weight-average molecular weights ($1/M_w$) obtained from DNA profiles from alkaline sucrose gradients of untreated (c) and MMS-treated (t) normal human fibroblasts. Untreated cells were pulse-labeled for 20 min. Treated cells were exposed to 10^{-5} (●), 10^{-4} (△), or 10^{-3} (O) M MMS for 1 hr before pulse-labeling at 0.5, 2.5, 4.0, or 12 h for 25, 30, 25, or 25 min, respectively. Data obtained from Buhl and Regan (21).

Fig. 11. DNA profiles from alkaline sucrose gradients of normal human fibroblasts treated for 1 hr with 10^{-4} M MMS, pulse-labeled for 30 min at various times after treatment, as indicated, and then incubated for 18 hr before sedimentation. Sedimentation was for 75 min at 40,000 rpm. Data obtained from Buhl and Regan (23).

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Fig. 1

25325-4

Fig. 2

27411

Fig. 3

26184-2

Fig. 4

26000-1

Fig. 5

27412

Fig. 6

26819-1

Fig. 7

27413

a UV IRRADIATED DNA

b DNA OBSERVED AFTER PULSE-LABELED

c COMPLETED DNA SYNTHESIS

Fig. 8

27414

Fig. 9

27062-2

Fig. 10

27065-2

Fig. 11