Analysis of Interphase Chromosome Damage by Means of Premature Chromosome Condensation after X- and Ultraviolet-Irradiation

(cell fusion/DNA synthesis)

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ABSTRACT Sendai virus-mediated fusion between mitotic and interphase mammalian cells causes the rapid condensation of the interphase chromosomes into distinct structures, a process termed premature chromosome condensation. This phenomenon has been used to assess the immediate action of x-rays and ultraviolet light on the chromosomes of HeLa cells irradiated in the G1 phase of the life cycle. X-irradiation produces fragmented chromosomes; but even the most finely chopped fragments retain the condensed morphology characteristic of the premature chromosome condensation of unirradiated G1 cells. For doses up to about 1800 rads, the increase in the number of fragments is linearly related to the dose. One mean lethal dose (about 100 rads) yields a net increase of 10-15 fragments per G1 cell, which is considerably larger than previous estimates based on scoring of mitotic chromosomes. Incubation of irradiated cells produces a rapid (within 2 hr) reduction in the number of fragments, indicative of a rejoining process. The decrease in the number of pieces is not accompanied by unscheduled DNA synthesis detectable by radioautography. G1 chromosomes of cells irradiated with UV light in G1 phase are not appreciably fragmented but are elongated and attenuated so that they resemble the premature-chromosome-condensation chromosomes of unirradiated S-phase cells. Both the degree of "S-like" character attained by the G1 chromosomes in a cell and the percentage of the cell population displaying the G1 → S transition increase with the dose and incubation time after irradiation. Thus, in contrast to the immediate manifestation of damage from x-rays, the maximum induction of the "S-like" state does not occur until about 2 hr after irradiation. The "S-like" chromosomes are capable of unscheduled DNA synthesis. We suggest that the difference in chromosome morphology found after UV- and x-irradiation underlies the reason why the former, but not the latter, induces unscheduled DNA synthesis in G1 cells.

A frequent consequence of the Sendai virus-mediated fusion between mitotic and interphase mammalian cells is the induction of the interphase nucleus into a mitotic state in which the chromosomal material condenses and becomes visible (1). The morphology of the induced condensation, termed premature chromosome condensation (PCC), depends on the stage in the cell cycle occupied by the interphase nucleus at the time of fusion. Thus, G1-PCC chromosomes consist of single strands; G2-PCC of mitotic-like, but elongated, bivalent structures; and S-PCC of a highly elongated network of intermingled thick and thin filaments (1, 2). Although the morphology of each type of PCC is distinctive, evidence exists that PCC is the interphase counterpart of mitotic chromosome condensation; e.g., the number of condensed interphase elements equals the number of mitotic chromosomes (3, this paper) and the G- and C-banding patterns are similar to those found in the homologous mitotic chromosomes (4). We therefore believe that an examination of PCC reveals the organization of the interphase genetic material.

In this study, the techniques of PCC reveal that chromosomes of HeLa cells x-irradiated in G1 phase differ in morphology and in DNA synthetic capacity from those exposed to UV. New determinations are made of the chromosome-breaking efficiency of x-irradiation, and some aspects of repair of x-ray and UV-light damage are discussed.

MATERIAL AND METHODS

HeLa cells with a modal chromosome number of 64 were cultivated in suspension and synchronized in mitosis by application of a nitrous oxide block (5, 6). Highly synchronous cells in early G1 phase (greater than 95%) were obtained by releasing the pressure block and allowing the mitotic cells to progress into interphase. Cells were x- and UV-irradiated at room temperature in growth medium (Eagle's minimal essential medium) supplemented with 5% fetal calf serum. X-irradiation was administered at 200 rads/min, 230 kV, 15 mJ/m²; UV light at 9 erg/mm² per sec, Phillips germicidal lamp emitting predominantly at 265 nm. After irradiation G1 cells were (a) immediately fused with mitotic cells; (b) plated in petri dishes in growth medium with or without [3H]dT (3 μCi/ml, 22 Ci/mmol) for 2 hr. Then all the cells were used for fusion by standard methods (6), with a ratio of 2:1 mitotic to interphase cells and 200 hemagglutinating units (HAU) of UV-inactivated Sendai virus. Colcemide, 0.25 μg/ml (Ciba Ltd.), was added to the fusion mixture to disrupt the spindle of the mitotic cells and enhance spreading of chromosome preparations. Chromosome spreads were prepared by swelling the cells in hypotonic saline and dropping them onto wet slides (7). Ilford G5 emulsion was used for radioautographs after the preparations had been hydrolyzed for 2 min at 60°C in HCl and exhaustively extracted with ice-cold 5% trichloroacetic acid to remove soluble radioactive products. Slides were exposed for three weeks and stained with toluidine blue. Slides for immediate cytological examination were hydrolyzed as above in hot HCl and stained in 5% crystal violet.

RESULTS

Effect of X- and UV-irradiation on the Morphology of G1 Chromosomes. X-rays chop chromosomes into fragments,
FIG. 1. (a) Unirradiated HeLa G1-PCC with 69 chromosomes (×2250). (b) Massively fragmented G1-PCC from a cell irradiated with 8000 rads and immediately fused with a mitotic cell to display the chromosomes. Very little continuity can be seen between the fragments (×2250). (c) G1-PCC with about 230 fragments; the pieces are considerably longer than in (b). Fusion immediately after 800 rads of x-irradiation (×2250). (d) G1-PCC with about 150 fragments. The length of chromosome fragments is greater than in (c). Fusion immediately after 200 rads of x-irradiation (×2250).
the number of which increases with the dose. To quantitate the immediate damage to G1 cells, we visualized the chromosomes in the form of PCC as quickly as possible after irradiation. Chromosome slides were systematically scanned, and an estimate was made of the number of fragments in each of 300 scorable spreads. Depending on the number of fragments it contained, a cell was placed into one of four arbitrarily defined categories (Table 1). A fragment was scored as one piece only when it was separated from any other. If any continuity, however tenuous, was present among apparently separate segments, the unit was counted as a single fragment. It is clear that the amount of fragmentation increases with the dose (Table 1).

To relate quantitatively the number of breaks per set of G1 chromosomes to the x-ray dose, we made exact counts of the number of fragments present in the first 30 cell spreads located in which the chromosome fragments were sufficiently distinct to permit enumeration. These data (Table 3 and Fig. 3) show that the net number of fragments per G1 cell increases linearly with the dose up to 1800 rads. The decreased slope of the curve at higher doses probably results from our inability to distinguish and accurately count the large number of tiny pieces.

**UV light.** The immediate effect of UV light on G1 chromosomes is to cause lengthening, diffusion, and attenuation rather than extensive breakage. The morphology of UV-PCC ranges from that of unirradiated G1 (Fig. 1a) to almost S phase (Fig. 2c). Both the degree of “S-like” character in an individual cell and the percentage of the total population with “S-like” PCC increase with the dose and time after irradiation (Table 2). Thus, the maximum induction of the “S-like” state requires a few hours’ incubation after irradiation, while the chopping action of x-rays is manifest immediately. Although the morphology of UV-induced “S-like” PCC resembles true S-PCC the two forms are distinguishable, since the chromatin threads are more discernible and thicker in the former (compare Fig. 2b with 2a).

**Repair: Evidence for rejoining of G1 chromatids fragmented by x-irradiation.** Tables 1 and 3 and Fig. 3 show the number of G1 chromosome fragments per nucleus present immediately after irradiation and after 2 hr of incubation. The number of pieces decreases during incubation and, although precise measurements have not been made, those remaining appear to have in-

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**Table 1. Percentage distribution of the number of chromosome fragments in G1 HeLa cells scored immediately after x-irradiation**

<table>
<thead>
<tr>
<th>X-ray dose* (rads)</th>
<th>X-ray dose* (rads)</th>
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<tr>
<td>0</td>
<td>92</td>
<td>8</td>
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<td>200</td>
<td>35</td>
<td>64</td>
<td>1</td>
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<td>800</td>
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<td>0</td>
<td>11</td>
<td>69</td>
</tr>
<tr>
<td>8000</td>
<td>0</td>
<td>5</td>
<td>15</td>
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</table>

* Three hundred chromosome spreads containing PCC were scored for each dose of x-irradiation.

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Fig. 2. (a) Unirradiated HeLa S-PCC after fusion between a mitotic HeLa cell and a cell in early S phase (×2250). (b) “S-like” G1-PCC after UV-irradiation of G1 HeLa. The chromosomes have diffused into a network of fine filaments (×2250). (c) Radioautograph of UV-irradiated (1600 ergs/mm²). G1 chromosomes after incubation in medium containing [3H]dT. The irradiated chromosomes are moderately attenuated, and show incorporation (×2250).
creased in length. Using radioautography we find that the reduction in the number of fragments occurs without detectable DNA synthesis, even at the highest dose of x-ray. In general, this result agrees with biochemical and radioautographic data from other laboratories (8).

**Evidence for DNA synthesis in UV-irradiated G1 cells.** The radioautograph shown in Fig. 2c demonstrates that UV-irradiated G1 chromosomes synthesize DNA. Incorporation of [3H]dT was found in chromosomes where the “S-like” morphology was evident to a greater or lesser degree, although grain density was highest in those “S-like” PCC with the greatest attenuation. At the highest dose used (about 1600 erg/mm²) some incorporation was present in a majority of distinguishable chromosomes irrespective of their morphology. Over 90% of the “S-like” PCC spreads were labeled. The irradiated G1 cells in the population that had not fused also incorporated [3H]dT into DNA. No incorporation of [3H]dT was found in the unirradiated G1-PCC.

**DISCUSSION**

These results show that it is now possible to visualize and quantitate the action of ionizing and nonionizing radiation on the chromosomes of interphase nuclei of mammalian cells. The effects on mammalian cells of these two types of irradiation are similar in many respects but different in others. For example, at mitosis, both irradiations efficiently produce chromatid and chromosome breaks, rearrangements, and chromatid exchanges (9). Both are highly lethal, and their killing action probably results from damage to chromosomes (10, 11). Ultraviolet-irradiation is two- to eight-fold more efficient than x-irradiation in the production of auxotrophic mutants in Chinese hamster cells (9).

A well-characterized difference in cell response to x- and UV-irradiation is the induction by the latter of substantial DNA synthesis in interphase G1 and G2 nuclei (12). This synthesis, termed unscheduled or repair synthesis, has been found in mammalian cells after low doses of UV light but is difficult, though not impossible, to demonstrate in x-irradiated cells (13, 14).

This paper demonstrates a new difference in response that may underlie the first—namely, the contrasting form of G1-PCC chromosomes produced by x-rays and UV light. Ultraviolet light drives G1 chromosomes into an “S-like” state that is morphologically and synthetically similar to the S phase. We propose that a consequence of this change in form is the production of a chromatin template suitable for DNA synthesis. Thus UV-induced DNA synthesis results from the cells’ entrance into a state that is in some respects comparable with the S phase. X-irradiation fragments chromosomes without changing them into a state capable of supporting DNA synthesis. Even the most finely chopped fragments retain the condensed morphology characteristic of unirradiated G1 chromosomes, and no incorporation of [3H]dT by these fragments is detectable. These data support the hypothesis that only a few bases are inserted per break (15).

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**Table 2. Percentage of PCC in each morphological category at t₁ = 0 hr and t₂ = 2 hr after UV-irradiation**

| UV dose (erg/mm²) | G1 | Intermediate between S and G1 | “S-like”
<table>
<thead>
<tr>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>t₁</td>
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</tr>
<tr>
<td>1620</td>
<td>69</td>
<td>18</td>
<td>11</td>
</tr>
</tbody>
</table>

* Three hundred chromosome spreads containing PCC were scored for each dose of irradiation and each time.

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**Table 3. Number of G1 chromosome fragments in HeLa cells immediately and 2 hr after x-irradiation**

<table>
<thead>
<tr>
<th>x-ray dose (rads)</th>
<th>t₀</th>
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<th>t₀</th>
<th>t₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range*</td>
<td>Total fragments†</td>
<td>Net fragments</td>
<td>Range*</td>
</tr>
<tr>
<td>0</td>
<td>42-72</td>
<td>64 ± 4</td>
<td>0</td>
<td>60-117</td>
</tr>
<tr>
<td>200</td>
<td>65-127</td>
<td>96 ± 6</td>
<td>32</td>
<td>82-208</td>
</tr>
<tr>
<td>800</td>
<td>102-200</td>
<td>143 ± 9</td>
<td>79</td>
<td>82-208</td>
</tr>
<tr>
<td>1800</td>
<td>145-433</td>
<td>240 ± 10</td>
<td>175</td>
<td>105-253</td>
</tr>
<tr>
<td>5000</td>
<td>147-460</td>
<td>318 ± 17</td>
<td>254</td>
<td>124-359</td>
</tr>
<tr>
<td>8000</td>
<td>224-250</td>
<td>404 ± 35</td>
<td>340</td>
<td>296-475</td>
</tr>
</tbody>
</table>

* The total number of G1-PCC fragments was determined in each of 30 G1-PCC chromosome spreads.† Standard deviation of the mean.
After UV-irradiation we observed virtually no chromosome breakage, yet this agent is known to produce such lesions as scored at mitosis. This disparity could be explained in at least three ways: (1) UV-induced breaks are not visible by our techniques; (2) the breaks are not manifest until some later stage in the life cycle; (3) the process of repair may itself be responsible for generating breaks that are seen at mitosis. Repair enzymes in mammalian cells can cause breaks in DNA (16).

Many attempts have been made to determine the number of chromosome breaks per cell caused by x-irradiation. However, the assessment was inaccurate because lesions are not visible until the subsequent mitosis, by which time a number may have been restored. Furthermore, a proportion of the more heavily damaged cells may never reach mitosis and so would not be scored. Various chemicals (17) have been used to prevent restitution of breaks, but their effectiveness is difficult to judge and scoring must still wait until mitosis. The number of chromatic lesions per mean lethal dose of x-irradiation (100-200 rads) delivered to a random population of cells, and remaining at mitosis, has been estimated at 1-3 (9, 17). Caspersson et al. found about three aberrations per cell, including reciprocal translocations and paracentric inversions, in metaphase plates of human lymphocytes irradiated with 226 rads during G0 or G1 phase (19). Because of the scoring difficulties mentioned, all of these values are acknowledged as minimal.

We find that a direct count of G1 chromatic fragments yields values about three- to ten-fold greater than those found previously at mitosis. This difference suggests three possibilities: (1) The more damaged cells may never reach mitosis; (2) our methods of preparation and scoring overestimate the number of pieces present; (3) substantial rejoining of fragments has occurred before the cells reach mitosis. The first point cannot be ruled out and may account in part for the low number of breaks seen in mitotic cells. As for the second point, the scoring was done as carefully as possible. While it is conceivable that the breaks produced are in part due to the procedures used, there is no reason to suspect these results any more than those found by methods of break-scoring in mitotic chromosomes. As for point three, our data on the reduction in the number of fragments after a 2 hr incubation support the conclusion that lesions are rejoined or restituted. Most fragments probably rejoin correctly so that no visible aberrations are seen at mitosis while other fragments may associate incorrectly to produce translocations, inversions, and other aberrations. The frequency of occurrence of such aberrations is dose-dependent (9, 18).

We find a net increase of about 10 fragments per cell per mean lethal dose, 100 rads (10); i.e., an increment of 1 fragment per 10 rads (Fig. 3). Thus, at least one break per cell is produced by 10 rads of x-irradiation administered to a G1 cell. Up to about 1800 rads, the net increase in the number of fragments is a linear function of the dose and appears to extrapolate to zero, indicating that significant chromosomal damage results from exposure to exceedingly low doses of ionizing radiation. These breakage values are minimal since many, perhaps most, of the lesions may rejoin during the 30 min required for visualization of chromosomes as PCC. The reduction in number of fragments during incubation could be trivially explained if the most damaged cells were unable to fuse after incubation. This seems unlikely since the frequency of induction of PCC was the same at the two times of fusion and was not reduced at high doses of x-ray.

The present studies demonstrate the usefulness of premature chromosome condensation in radiation biology and in studies related to the normal progress of G1 cells into the state of DNA synthesis.

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