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Genetic Heterogeneity in Xeroderma Pigmentosum: Complementation Groups and Their Relationship to DNA Repair Rates

(ultraviolet radiation/mutations/unscheduled DNA synthesis/somatic cell genetics)

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ABSTRACT **Fibroblast strains from 12 patients with xeroderma pigmentosum had lower than normal rates of DNA repair, as determined by autoradiographic studies of ultraviolet-induced unscheduled nuclear DNA synthesis. The nuclei in binuclear cells, obtained by fusing fibroblasts from certain pairs of these strains, had a greater rate of DNA repair than the nuclei of either strain's unfused mononuclear cells. These results indicate that complementary corrections of the strains' repair defects had occurred in the fused cells. Four complementation groups were found, indicating that at least four mutations caused decreased DNA repair among these 12 strains. The unfused mononuclear cells of each group had a characteristic rate of repair that differed from the rates of the other groups.**

In xeroderma pigmentosum (XP), a rare disease of autosomal, recessive inheritance, sun-exposed skin develops severe solar damage, pigmentation changes, and neoplasms (1). XP cells repair DNA damaged by ultraviolet (UV) light more slowly than normal cells (1–3). Defective repair in cells from most XP patients can be detected by measuring the rate at which the cells incorporate [^{3}H]thymidine into DNA segments synthesized to replace regions containing UV-induced pyrimidine dimers (1, 2, 4). This [^{3}H]thymidine incorporation reflects DNA repair replication and is manifested autoradiographically as unscheduled DNA synthesis (1, 2, 4–6).

XP is one of the few human diseases (7, 8) in which somatic cell genetic studies have successfully shown different mutations to be responsible for an observed phenotype. When fibroblast strains from certain pairs of XP patients are fused in culture to form binuclear heterokaryons, both of the nuclei in the fused cells perform more unscheduled DNA synthesis than the nuclei of the unfused mononuclear cells of either strain (1, 9). De Weerd-Kastelein *et al.* (9) first demonstrated this genetic heterogeneity in XP and have found three complementation groups (10). We have found four complementation groups among the 12 XP strains we have studied, and we have shown that all the strains within a complementation group have a similar rate of unscheduled DNA synthesis that is characteristic for each group.

MATERIALS AND METHODS

Fibroblast Strains. Twelve XP and three normal control donor strains were from the American Type Culture Collection, Rockville, Md. Ten of the XP strains were from patients of the NIH series (1). These XP strains and strains XPKMSF (11) and XP1LO (12) are identified by the nomenclature for XP strains (13). Fibroblasts were grown without antibiotics in modified Ham's F12 medium (14) supplemented with 5% fetal calf serum at 37° in a 5% CO_2–95% air incubator with more than 95% humidity. They were used after two to seven additional passages.

Cell Fusion. One- to 3-day-old coverslip cultures in 60-mm petri dishes were used for irradiation and fusion, the latter performed by a modification of the method of Yamanaka and Okada (15). Cultures, cooled on ice, were washed with cold Hanks' balanced salts solution and covered with 0.1 ml of cold, serum-free medium containing about 100 hemagglutinating units [standardized with 0.03% (v/v) chick erythrocytes] of β-propiolactone-inactivated Sendai virus (14). Ten minutes later, about 0.1 ml of cold, serum-free medium containing 5×10^4 cells (to be fused with those already on the coverslips) was dropped on each coverslip. After 20 min in the cold, the dishes were placed in the 37° incubator. Three hours later the coverslips were covered with serum-containing medium (3 ml per dish), and the incubation was continued.

UV Irradiation. About 16 hr after addition of inactivated virus, the cultures were washed twice with phosphate-buffered saline (pH 7.4) at room temperature and covered with phosphate-buffered saline (1 ml per dish). Immediately after irradiation with 300 erg/mm^2 of 254 nm UV light (16), the phosphate-buffered saline was removed and 2 ml of Hanks' base medium 199 (Difco Laboratories or NIH Media Unit) containing bicarbonate, 20% fresh, heparinized, filtered human plasma, and 20 μCi of [*methyl*-^{3}H]thymidine (specific activity, 17–25 Ci/mmol; Amersham-Searle) was added. The dishes were placed in the incubator for 3 hr. The cultures were then washed and fixed. Unirradiated coverslip cultures were processed in parallel with the irradiated cultures.

Autoradiograms and Their Analysis. Autoradiograms, prepared with NTB-3 emulsion (Eastman Kodak), were exposed at 4° for 7 days (8 days in the case of Exp. 1 of Table 1), then developed and stained. Grain production and counting efficiencies were constant for the range of grain counts in these experiments.

On coverslip cultures not treated with virus, grains over "lightly labeled" (1, 2, 5, 6) nuclei from 100 consecutively observed mononuclear cells were counted. Virus-treated coverslips were scanned under low power for areas containing multinucleate cells that had the most light labeling. In such an area, grains were counted over the 100 nuclei of 50 consecu-

Abbreviations: XP, xeroderma pigmentosum; UV, ultraviolet.

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TABLE 1. *Relationship of the XP complementation groups to the amount of UV-induced unscheduled DNA synthesis performed by XP fibroblast strains during the first 3 hr after irradiation*

Complementation group	Fibroblast strain	% of Normal unscheduled DNA synthesis* (mean grain count)†				
		Exp. 1‡	Exp. 2‡	Exp. 3‡	Exp. 4‡	Exp. 5‡
	Control donor R	100 (114.8)	100 (117.2)	—	—	—
	Control donor L	100 (114.9)	—	100 (125)	—	—
	Control donor P	—	—	—	100 (130.2)	100 (99.9)
A	XP1LO	<2 (7.2)	<2 (5.9)	—	—	—
A	XPKMSF	<2 (5.8)	—	—	—	—
A	XP12BE	—	—	<2 (3.6)	<2 (3.6)	<2 (4.2)
B	XP11BE	7 (12.6)	7 (11.7)	—	3 (6.3)	6 (8.3)
C	XP1BE	20 (27.0)	—	—	—	—
C	XP2BE§	16 (22.5)	—	18 (23.4)	13 (17.8)	17 (19.0)
C	XP3BE	12 (17.3)¶	—	—	—	—
C	XP8BE§	—	—	—	—	15 (16.7)
C	XP10BE	13 (18.5)	—	—	—	—
D	XP5BE‖	31 (39.2)	46 (56.4)	—	—	—
D	XP6BE‖	—	—	—	—	47 (47.9)
D	XP7BE	31 (38.9)	24 (31.7)	—	—	—

* Values were calculated for strains in groups B, C, and D by dividing the UV-induced unscheduled DNA synthesis of the irradiated cells by the UV-induced unscheduled DNA synthesis of the control donor's irradiated cells and multiplying the quotient by 100. The UV-induced unscheduled DNA synthesis is the difference between the mean grain count per nucleus for the irradiated cells in an experiment (in parentheses in the Table) and the average nuclear background for that experiment (see footnote‡). Whatever UV-induced unscheduled DNA synthesis group A fibroblasts might have, it does not exceed an average of 2.0 grains per nucleus and, hence, must be less than 2% of the control donors' UV-induced unscheduled DNA synthesis.

† This value is the mean grain count per nucleus for 50 control donor, or 100 XP, consecutively evaluated, lightly-labeled nuclei of irradiated mononuclear cells. For control donor L of Exp. 3, the mean grain count is calculated from 100 nuclei; for control donor P in Exp. 5, from 75 nuclei. Occasional cells with a high grain count that were apparently not in the grain distribution of the evaluated group were excluded from the computation of the mean grain count.

‡ The cultures used for these experiments were not treated with Sendai virus except in the one case noted below (footnote ¶). The nuclear background for each of these experiments is presented as the range (and average) of the mean grain counts per nucleus of n sets of 100 nuclei from unirradiated mononuclear cells as follows: Exp. 1, 1.9–6.9 (4.5), $n = 10$; Exp. 2, 2.5–6.7 (4.2), $n = 5$; Exp. 3, 1.1–2.4 (1.7), $n = 6$; Exp. 4, 1.0–2.3 (1.7), $n = 4$; Exp. 5, 2.1–2.9 (2.5), $n = 3$.

§ Patient XP2BE and patient XP8BE are brothers.

¶ Value obtained for irradiated mononuclear cells that escaped fusion in a Sendai virus-treated coverslip culture containing strain XP1BE and XP3BE fibroblasts. No virus-free coverslip cultures containing only XP3BE cells were prepared in any of these experiments.

‖ Patient XP5BE and patient XP6BE are sisters.

tively observed binuclear cells and over the nuclei of the first 100 consecutively observed mononuclear cells.

RESULTS

Each of the 12 XP fibroblast strains used has a reduced rate of UV-induced unscheduled DNA synthesis (Table 1). Group A strains had less than 2% of the normal rate, while the strains in groups B, C, and D had rates 3–7%, 10–20%, and 25–50% of normal, respectively.

Fig. 1 presents autoradiographic data for normal control donor fibroblasts and for an XP fibroblast strain from each of the four complementation groups. For the unirradiated strains shown (*a*, *c*, *e*, *g*, and *i*), more than 95% of the cells of each histogram had 15 or fewer grains per nucleus. The control donor's irradiated cells (*b*) all had more than 55 grains per nucleus, and their mean grain count (arrow) was 114.8 grains per nucleus. The irradiated XP cells from groups A (*d*), B (*f*), C (*h*), and D (*j*) had mean grain counts of 7.2, 12.6, 27.0, and 39.2 grains per nucleus, respectively.

Significant UV-induced unscheduled DNA synthesis could not be demonstrated in irradiated group A cells (Fig. 1*d*). However, the irradiated cells from groups B (*f*), C (*h*), and D (*j*) clearly show such synthesis. The unscheduled DNA synthesis of normal and XP mononuclear cells was not affected by virus treatment.

With our Sendai virus fusion technique, some mononuclear cells escape fusion while others fuse to form cells having two or more nuclei. If both nuclei in a binuclear cell are from the same strain, the cell is a homokaryon. If the nuclei are from different strains, the cell is a heterokaryon. When cells from certain pairs of our XP strains were fused and then studied for UV-induced unscheduled DNA synthesis, both the nuclei in some of the resulting binuclear heterokaryons (e.g., binuclear cell *f* in Fig. 2) had light labeling consisting of more grains per nucleus than the labeling over the unfused mononuclear cells on the same coverslips (cells *a–c*, Fig. 2). These binuclear cells had considerably fewer grains than those of the heavily labeled cells in S-phase DNA synthesis (cell *d*, Fig. 2). Such lightly labeled cells were considered to be complementing heterokaryons. Binuclear cells (cell *e*, Fig. 2) whose nuclei had no more labeling than the unfused mononuclear cells were assumed to be homokaryons. The light labeling of the nuclei in complementing heterokaryons was studied in the presence of hydroxyurea, which inhibits S-phase DNA synthesis while having no effect on unscheduled DNA synthesis of DNA repair replication (17). The light labeling of the complement-

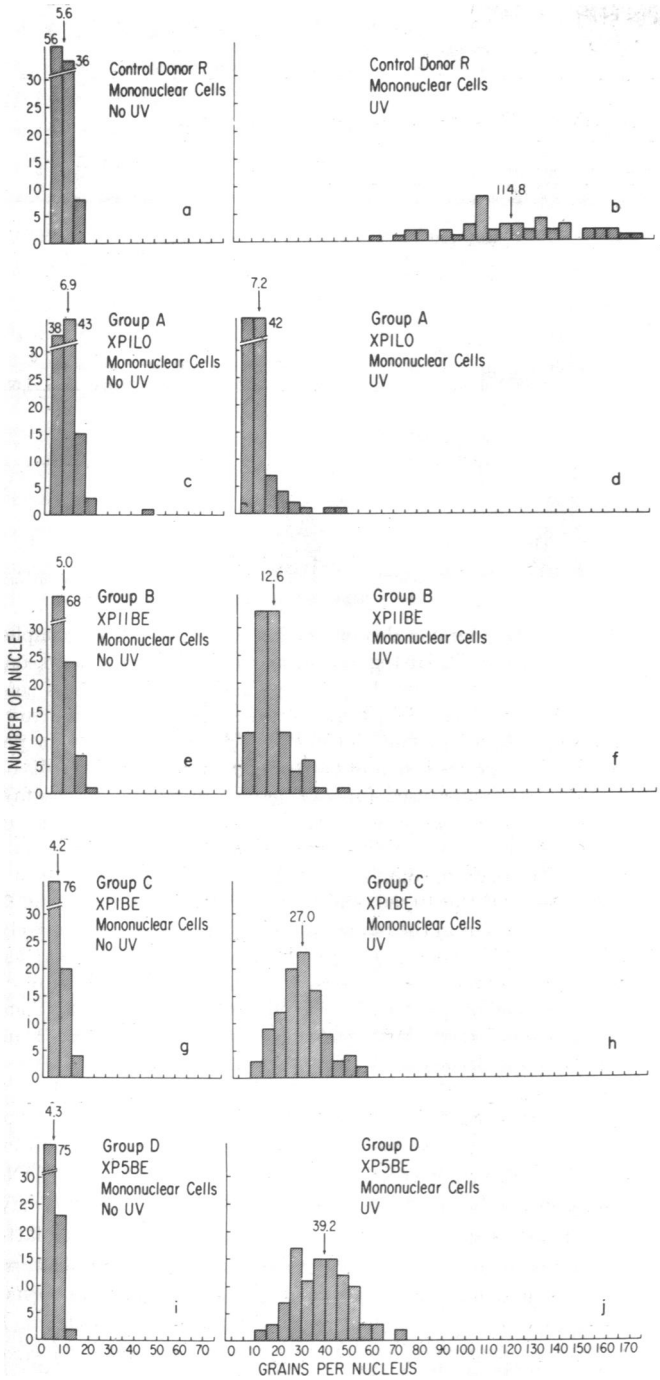

FIG. 1. Histograms showing UV-induced unscheduled DNA synthesis during the first 3 hr after UV irradiation with 300 erg/mm² in a fibroblast strain from a control donor and in a fibroblast strain from each of the four complementation groups. Data are from the autoradiograms of Exp. 1, Table 1. Histograms in left column (*a*, *c*, *e*, *g*, and *i*) show nuclear background for each strain's unirradiated cells. Histograms in the right column (*b*, *d*, *f*, *h*, and *j*) show nuclear background plus any UV-induced unscheduled DNA synthesis for each strain's irradiated cells. The grain class of 0 through 5 grains per nucleus includes nuclei with no grains as well as those with as many as 5 grains; grain classes then run successively as 6 through 10, 11 through 15, etc. When more than 35 nuclei are in a single grain class, the bar representing them is truncated, and the number of nuclei in that class is printed near the top of the bar. Each arrow indicates the mean grain count for the 100 consecutively evaluated nuclei of each histogram (50 in the case of *b*) except that occasional cells with an unrepresentatively high grain count were excluded.

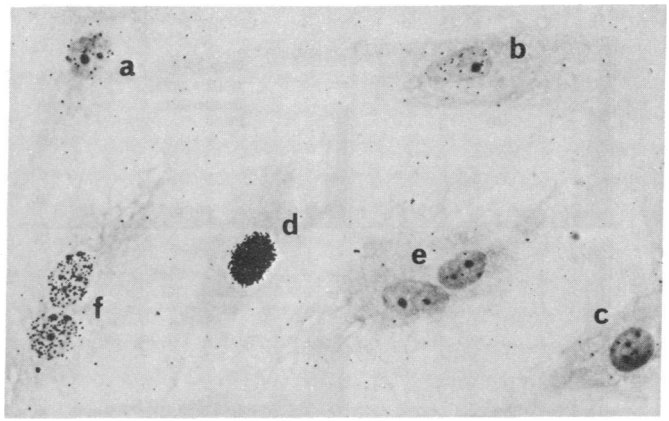

FIG. 2. Autoradiograms of a Sendai virus-treated culture of two complementing XP fibroblast strains, XP1BE (group C) and XP12BE (group A). The cells were irradiated and incubated with [³H]thymidine as described in *Materials and Methods*. Cells *a–d* are unfused mononuclear cells; *e* and *f*, binuclear cells. Cells *a* and *b* show unscheduled DNA synthesis typical of strain XP1BE (Table 1 and Fig. 1*h*); *c* and *e* have low grain counts typical of XP12BE (Table 1); *d* is "heavily labeled" in S-phase synthesis; *f* is a complementing heterokaryon. ×400.

ing cells was not noticeably decreased by 10 mM hydroxyurea, a concentration that inhibited S-phase DNA synthesis in the cultures by more than 95%.

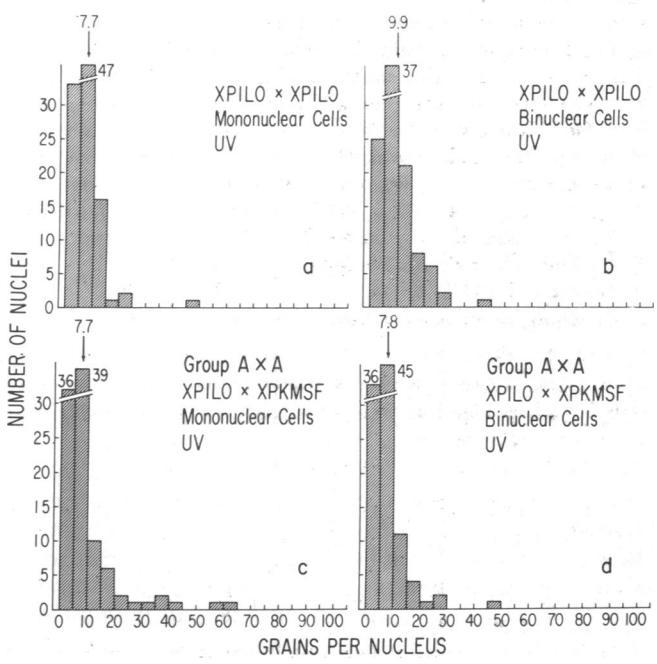

FIG. 3. Histograms for Sendai virus-treated, irradiated fibroblasts showing lack of complementation when XP fibroblasts are fused with cells from the same strain or with cells from another strain in the same complementation group. *a*, Unfused mononuclear cells on a coverslip containing cells only from strain XP1LO. *b*, Binuclear cells (homokaryons) on the same coverslip. *c*, Unfused mononuclear cells on a coverslip containing cells from both strains XP1LO and XPKMSF. *d*, Binuclear cells (homokaryons of XP1LO and of XPKMSF; and heterokaryons with a nucleus from each strain) on the latter coverslip. Each arrow indicates the mean grain count for the nuclei with 30 or fewer grains per nucleus among the 100 consecutively observed nuclei in each histogram. Histograms are presented as in Fig. 1 and *Materials and Methods*.

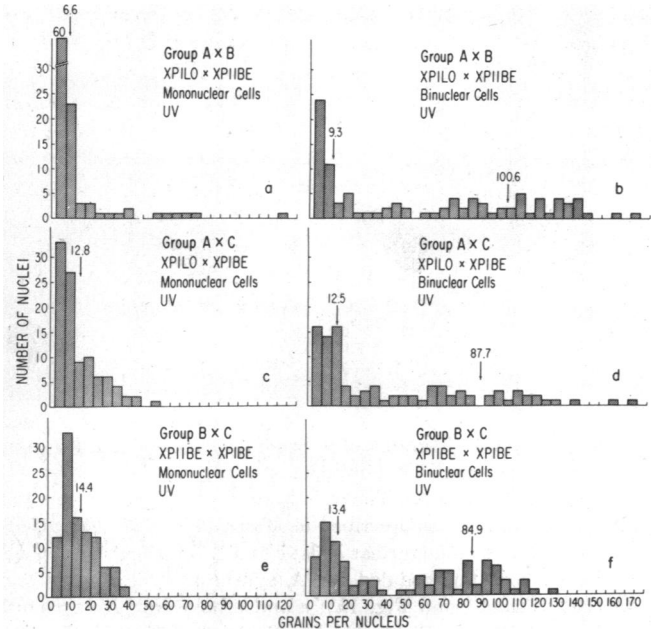

FIG. 4. Histograms demonstrating existence of complementation groups A, B, and C among three XP fibroblast strains. Histograms *a*, *c*, and *e* are of irradiated, unfused mononuclear cells that escaped fusion on coverslips containing Sendai virus-treated cells of the strains indicated. Each arrow indicates the mean grain count for the nuclei with 40 or fewer grains per nucleus except for *c*, where all the cells were included. Histograms *b*, *d*, and *f* are of irradiated binuclear cells on the same coverslips used in *a*, *c*, and *e*, respectively. The first arrow in each histogram indicates the mean grain count for the nuclei with 40 or fewer grains per nucleus; the second arrow indicates the mean grain count for the nuclei with more than 40 grains per nucleus. In *b* there were 52 of the latter nuclei, of which 92% were accompanied by another nucleus with more than 40 grains per nucleus; in *d*, of the 40 such nuclei, 90% were so accompanied; in *f*, all 50 of such nuclei were so accompanied. Histograms are presented as in Fig. 1 and *Materials and Methods*.

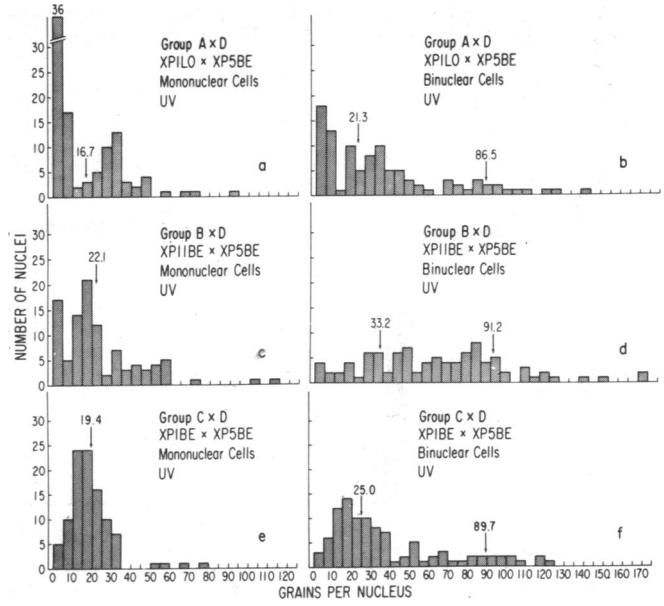

FIG. 5. Histograms demonstrating the existence of complementation group D. Histograms *a*, *c*, and *e* are of irradiated, unfused mononuclear cells that escaped fusion on coverslips containing Sendai virus-treated cells of the strains indicated; arrows indicate mean grain counts for nuclei with 60 or fewer grains per nucleus. Histograms *b*, *d*, and *f* are of irradiated binuclear cells on the same coverslips used for *a*, *c*, and *e*, respectively; the first arrow in each histogram indicates the mean grain count for the nuclei with 60 or fewer grains per nucleus; the second arrow indicates the mean grain count for the nuclei with more than 60 grains per nucleus. Of the 19, 50, and 21 nuclei that had more than 60 grains per nucleus in the binuclear cells of *b*, *d*, and *f*, respectively, 63, 80, and 67% were accompanied by another nucleus that had more than 60 grains per nucleus. Autoradiographic exposure was 8 days throughout, except for the coverslip for histograms *c* and *d*, which was 7 days. Histograms are presented as in Fig. 1 and *Materials and Methods*.

Complementation was not found in binuclear cells containing nuclei from the same strain. For example, on a coverslip containing virus-treated, irradiated cells only from strain XP1LO, the mean grain count and the grain distribution pattern for the binuclear cells (Fig. 3*b*) were similar to those for the mononuclear cells on the same coverslip (Fig. 3*a*), and no new population of nuclei with more than 40 grains per nucleus appeared among the binuclear cells. In such a fusion all the multinucleate cells formed would be homokaryons. Complementation was also not seen after fusion of cells from certain pairs of strains (see, e.g., Fig. 3*c* and *d*). Some of the binuclear cells formed in such fused mixtures would be heterokaryons; however, since no complementing nuclei could be found in any of the binuclear cells from such fused strain pairs (Fig. 3*d*), we consider such strains to be noncomplementing, i.e., in the same complementation group.

The existence of three complementation groups, A, B, and C, can be demonstrated by studying the UV-induced unscheduled DNA synthesis in Sendai virus-treated mixed cultures resulting from the three possible pairings between strains XP1LO, XP11BE, and XP1BE. Each of these three strains complements the other two strains, as shown by the presence in some of the binuclear cells (the presumed heterokaryons) of populations of lightly labeled nuclei with more than 40 grains per nucleus (Fig. 4*b*, *d*, and *f*). Of these nuclei, 90% or more were in binuclear cells in which both nuclei had more than 40 grains per nucleus, indicating that a mutual, complementary correction of the impaired unscheduled DNA synthesis in both nuclei had occurred. These nuclei have grain class distributions and mean grain counts (the latter indicated by the second arrows in Fig. 4*b*, *d*, and *f*) that approach the distribution and mean grain count for the normal, irradiated, unfused mononuclear cells (Fig. 1*b*). The nuclei in the binuclear cells that had fewer than 40 grains per nucleus (the presumed homokaryons of each of the strains present) (Fig. 4*b*, *d*, and *f*) have mean grain counts (the first arrows) and grain class distributions essentially similar to those of the nuclei in the mononuclear cells on the same coverslips (Fig. 4*a*, *c*, and *e*).

Fig. 5 illustrates the existence of complementation group D. When XP5BE cells were fused with XP1LO (group A), XP11BE (group B), or XP1BE (group C) cells, complementing populations of nuclei appeared in the binuclear cells (Fig. 5*b*, *d*, and *f*).

Table 2 shows the results of complementation testing of the 12 XP fibroblast strains studied. Each strain falls into one of the four complementation groups, A, B, C, or D. Strains within a group do not complement any other strain within the same group. As expected, siblings (XP2BE and XP8BE in group C; XP5BE and XP6BE in group D) are in the same complementation groups.

TABLE 2. *Assignment of the 12 XP fibroblast strains to their unscheduled DNA synthesis complementation groups**

	XP1LO	XPKMSF	XP12BE	XP11BE	XP1BE	XP2BE	XP3BE	XP8BE	XP10BE	XP5BE	XP6BE	XP7BE
XP1LO	0											
XPKMSF	0	n										
XP12BE	0	0	0									
XP11BE	+	+	+	0								
XP1BE	+	+	+	+	0							
XP2BE	+	+	+	+	0	0						
XP3BE	+	n	+	+	0	0	0					
XP8BE	n	n	+	+	0	0	0	n				
XP10BE	+	n	+	+	0	0	0	0	0			
XP5BE	+	+	n	+	+	+	+	n	+	0		
XP6BE	n	n	+	+	+	n	n	+	+	0	n	
XP7BE	+	+	+	+	+	+	+	n	+	0	0	0

* +, Complementing; 0, noncomplementing; n, not studied. Fibroblast strains listed in the vertical columns were fused to those listed in the horizontal columns. Complementation was determined autoradiographically by visual inspection (e.g., see Fig. 2) or by grain count analysis (as in Figs. 3–5).

DISCUSSION

The light labeling seen after UV-induced unscheduled DNA synthesis in unfused, mammalian mononuclear cells (5, 6), including XP cells (1–3, 11, 16, 18), reflects the insertion of radioactive nucleosides during hydroxyurea-resistant (17) repair replication into gaps created by excision of DNA segments containing UV-induced pyrimidine dimers. That the UV-induced unscheduled DNA synthesis we observed in complementing binuclear XP fibroblasts represents DNA repair replication is supported by resistance of the unscheduled DNA synthesis to hydroxyurea and by experiments that indicate that such complementing, fused XP cells have normal repair replication (19), thymine dimer excision (20), and host-cell reactivation of UV-damaged adenovirus 2 (R. S. Day, III, K. H. Kraemer, and J. H. Robbins, unpublished data).

Each of our four complementation groups has a characteristic rate of UV-induced unscheduled DNA synthesis given by the unfused mononuclear cells of its strains (Table 1). A similar relationship has been found (13, 21) for the three groups of De Weerd-Kastelein *et al.* (9, 10). In experiments to relate their three complementation groups with our groups, fusion studies in their laboratory (21) and in ours have revealed that only one of their three groups, the one containing their strain XP2RO, is different from all of our four groups. Thus, there are five known complementation groups in XP (21). Our method for evaluating unscheduled DNA synthesis shows that their strain XP2RO has a greater rate of unscheduled DNA synthesis than the strains in our group D, and the complementation group containing strain XP2RO has, therefore, been named group E.

The only other human disease in which cell fusion studies have revealed complementation groups relating to a defective biochemical process that can be quantitated in the unfused cells is maple syrup urine disease (8). In this disease, however, unlike XP, the strains within a complementation group do not have similar degrees of reduced enzyme activity, and strains with apparently similar degrees are in different complementation groups (8). On the other hand, for mutations in the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) in cultured Chinese hamster cells, strains within one complementation group had, as for XP, a similar level of enzyme activity different from the level in the other complementation group (22).

Existence of five complementation groups in XP indicates that at least five mutations result in decreased rates of UV-induced unscheduled DNA synthesis. Attempts are being made to correlate each of these repair defects with abnormal functioning of XP cells. Cutaneous manifestations of XP, including neoplasms, are present in patients of all groups, but neurological abnormalities appear to be present only in patients of groups A, B, and D (1). The capacity of XP fibroblast strains to reactivate UV-damaged adenovirus is related to their complementation group assignment (23). Colony-forming ability after UV irradiation can differ among XP fibroblast strains (K. H. Kraemer, S. F. Barrett, and J. H. Robbins, unpublished data), and preliminary studies indicate that strains within a group have the same sensitivity to UV radiation. Thus, the identity of the complementation groups derived from studies of unscheduled DNA synthesis in fused cells appears to be preserved in relation to other UV-associated processes.

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