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Xeroderma pigmentosum complementation group H falls into complementation group D

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A number of human genetic syndromes are associated with defects in DNA-repair systems. One of the best characterized and most extensively studied is xeroderma pigmentosum (XP) (for a review see Kraemer et al., 1987). Patients suffering from this rare, autosomal recessive disorder develop solar damage and pigmentation abnormalities in the skin on sun-exposed areas. Sometimes the disease is associated with neurological defects. Furthermore, XP individuals display a marked increase in the frequency of skin cancer. The molecular defect in most patients is thought to reside in ineffective removal of DNA lesions by the excision-repair system. Extensive heterogeneity is found with respect to clinical symptoms, and cellular and biochemical parameters between different patients. This heterogeneity is also reflected at the genetic level by the identification of a large number of complementation groups, designated XP-A to XP-H (Moshell et al., 1983) in which the excision-repair system is defective, and a group designated XP-variant, in which a process termed post-replication repair is considered to be affected (Lehmann et al., 1975). Two exceptional patients have been described, both of them have the clinical symptoms of Cockayne's syndrome (CS, a distinct rare excision-repair disorder, Lehmann and Norris, 1989; Venema et al., 1990) as well as XP. One of them, patient XPCS1 or XP11BE, is the sole representative of complementation group B (Robbins et al., 1974), the other patient, XPCS2 (cell strain GM03248), first described by Lafforet and Dupuy (1978), was reported by Moshell and coworkers to constitute the new complementation group H (Moshell et al., 1983). These special cases demonstrate an overlap between two otherwise distinct DNA-repair syndromes. In a recent paper of Johnson and coworkers (1989), in which they presented the further characteristics of an XP-D-like HeLa-XP-D hybrid (HD2), the authors were unable to find complementation between this hybrid and XP-H cells. On the other hand, Fujiwara's group reported complementation between all strains they had previously assigned to XP complementation group D and GM03248 (XP-H) (Fujiwara and Satoh, 1985; Ichihashi et al., 1988). Because of the apparent confusion about the status of XP-H (see Robbins, 1989;

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Johnson, 1989) we decided to carefully reinvestigate the complementation analysis of XP-H and XP-D, the results of which are presented here.

Materials and methods

Cells, culture conditions and cell hybridization

A summary of the cell strains used is presented in Table 1. Unless specified otherwise all primary fibroblasts were grown on Ham's F-10 medium (Gibco) supplemented with 12% fetal calf serum (Biological Industries), penicillin (100 IU/ml) and streptomycin (100 μg/ml).

Each fusion partner was labeled with latex beads of different sizes (0.78 μm and 2.02 μm) by adding a suspension of beads to the medium of a subconfluent fibroblast culture 3 days prior to cell fusion (Matsukuma et al., 1981). Cell fusion was performed with the aid of inactivated Sendai virus as described by de Weerd-Kastelein et al. (1972) or using PEG4000 (Stefanini et al., 1986). The suspension of fused cells was seeded on coverslips and cultured 2 days prior to the complementation assay.

Complementation assay

Coverslips with fibroblast cultures after fusion were washed with phosphate-buffered saline (PBS), irradiated with UV-C light and assayed for UV-induced unscheduled DNA synthesis (UDS) essentially as described by Vermeulen et al. (1986). Briefly, immediately after irradiation (UV dose 15 or 20 J/m^2) the cells were incubated for 2 or 3 h in ^{3}H-labeled TdR, washed 3 times in PBS and fixed prior to the autoradiography procedure. After autoradiography the number of grains above nuclei of homodikaryons of each strain and of heterodikaryons was determined, providing a quantitative measurement of UV-induced DNA-repair synthesis in these cells. Complementation of 2 XP strains is indicated by induction of UDS to the level of repair-proficient fibroblasts assayed in parallel under the same conditions.

DNA fingerprint analysis

DNA isolated from 3 XP-H sublines (Table 1) and from an unrelated control (MK) was digested with the restriction endonuclease *Hin*fI, fragments were separated on a 0.8% agarose gel and transferred to nitrocellulose filters. The blot was hybridized with a minisatellite probe 33.15 (Jeffreys et al., 1985).

Results

Somatic cell hybridization experiments were performed between XPCS2-I, an XP-H primary fibroblast line used in our laboratory since 1982, obtained from Dr. Boué (Paris), and an XP-D reference strain XP1BR. As positive and negative controls XPCS2-I was also fused with XP25RO (a reference XP-A strain) and XP1BR was fused with another XP-D cell strain, XP6BE (for refer-

TABLE 1

INFORMATION ABOUT CELL STRAINS USED

Cell strain	XP complementation group	Remarks, reference
XP25RO	A	Reference XP-A strain, Kraemer et al. (1975)
XP13PV	C	Reference XP-C strain, Stefanini et al. (unpublished results)
XP1BR	D	GM03615, Camden Cell Bank repository
XP6BE	D	Reference XP-D strain, Kraemer et al. (1975)
XP3NE	D	Reference XP-D strain, De Weerd-Kastelein et al. (1976)
XP126LO	F	Reference XP-F strain, Norris et al. (1988)
XPCS2-I	H	Strain obtained in 1982 from Dr. Boué (Paris), in use in our laboratory since then, Lafforet and Dupuy (1978); Moshell et al. (1983)
XPCS2-II	H	Same as XPCS2-I, stored in liquid N_2 since its arrival in 1982
GM03248	H	Reference XP-H strain, Camden Cell Bank repository
C5RO, C1PV	human wild type	

TABLE 2

COMPLEMENTATION ANALYSIS OF XP-H AND XP-D FIBROBLASTS BY UDS COUNTING OF DIKARYONS

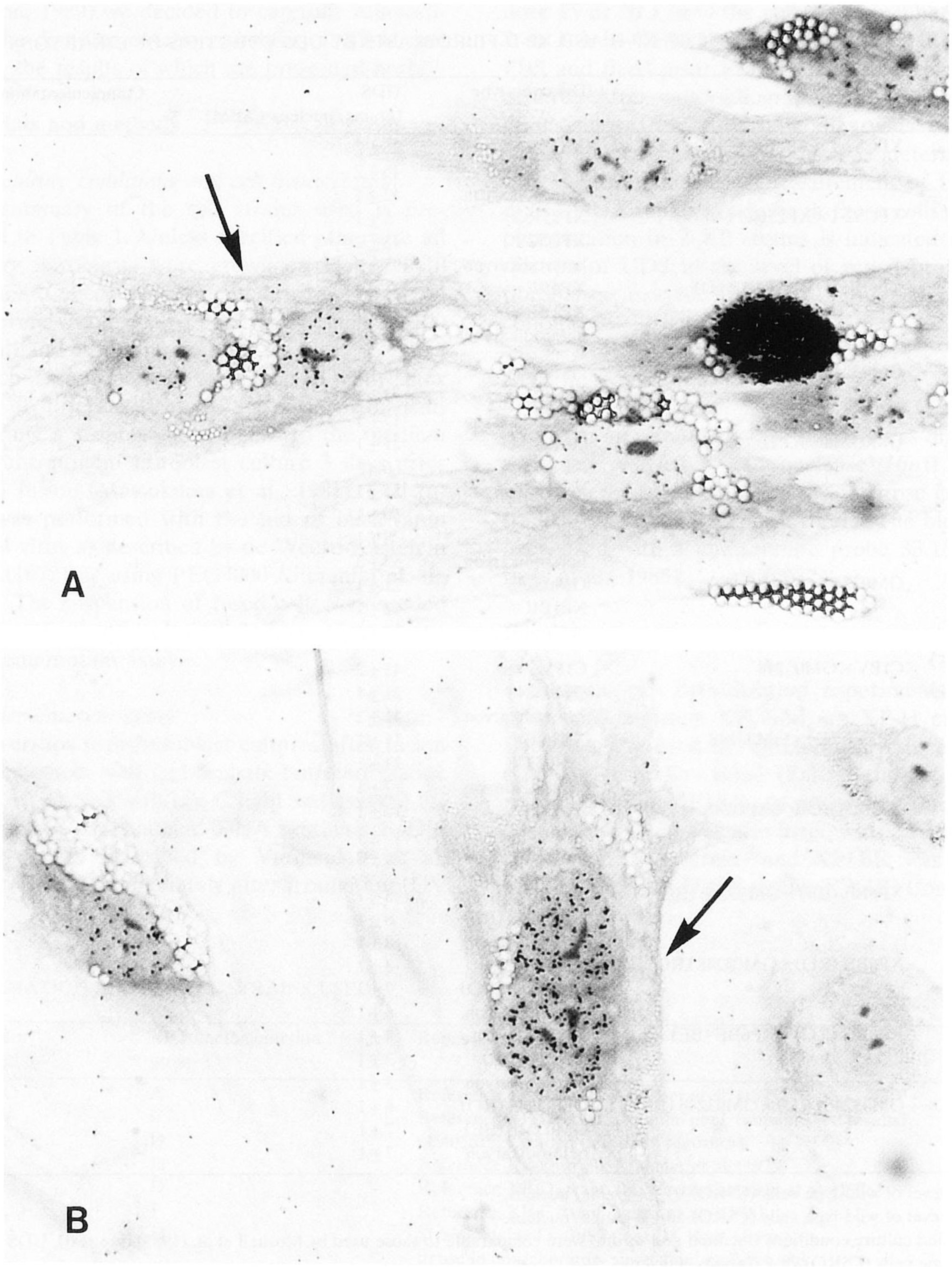
Expt.	Fused cells	Dikaryon type	UDS (grains/nucleus ± SEM)	Complementation
I [a]	XPCS2-I × XP1BR (D)	XP1BR	25 ± 2	
		XPCS2-I	46 ± 2	
		heterodikaryon	42 ± 1	–
	XPCS2-I × XP25RO (A)	XP25RO	5 ± 1	
		XPCS2-I	43 ± 2	
		heterodikaryon	95 ± 3	+
	XP6BE (D) × XP1BR (D)	XP6BE	21 ± 1	
		XP1BR	21 ± 1	
		heterodikaryon	20 ± 1	–
II [b]	GM03248 × XP6BE (D)	GM03248	18 ± 1	
		XP6BE	7 ± 1	
		heterodikaryon	17 ± 1	–
	GM03248 × XP126LO (F)	GM03248	21 ± 1	
		XP126LO	6 ± 1	
		heterodikaryon	51 ± 1	+
	XP6BE (D) × XP126LO (F)	XP6BE	10 ± 1	
		XP126LO	8 ± 1	
		heterodikaryon	53 ± 3	+
	GM03248 × XP1BR (D)	GM03248	21 ± 1	
		XP1BR	12 ± 1	
		heterodikaryon	21 ± 1	–
III	C1PV × GM03248	C1PV	41 ± 2	
		GM03248	21 ± 1	
		heterodikaryon	41 ± 2	+
	XP3NE (D) × GM03248	XP3NE	15 ± 1	
		GM03248	21 ± 1	
		heterodikaryon	17 ± 1	–
	XP13PV (C) × GM03248	XP13PV	12 ± 1	
		GM03248	17 ± 1	
		heterodikaryon	36 ± 2	+
IV [c]	XP6BE (BE) × GM03248 (BE)	XP6BE (BE)	3 ± 1	
		GM03248 (BE)	8 ± 1	
		heterodikaryon	8 ± 1	–
	XP6BE (RO) × GM03248 (RO)	XP6BE (RO)	3 ± 1	
		GM03248 (RO)	7 ± 1	
		heterodikaryon	6 ± 1	–
	XP6BE (RO) × XP6BE (BE)	XP6BE (RO)	4 ± 1	
		XP6BE (BE)	3 ± 1	
		heterodikaryon	3 ± 1	–
	GM03248 (RO) × GM03248 (BE)	GM03248 (RO)	8 ± 1	
		GM03248 (BE)	7 ± 1	
		heterodikaryon	7 ± 1	–

[a] UDS level of wild-type cells (C5RO) 93 ± 4 grains/nucleus.

[b] UDS level of wild-type cells (C5RO) 50 ± 2 grains/nucleus.

[c] Cells and culture conditions (medium and serum) were comparable to those used by Moshell et al. (1983) (see text), UDS level of wild-type cells (C5RO) 25 ± 1 grains/nucleus.

UV-dose for experiments I, II and IV, 15 J/m^2; for experiment III, 20 J/m^2. Incubation with ^{3}H-labeled TdR for experiments I, II and IV, 2 h, 10 μCi/ml, spec. act. 50 Ci/mmole; for experiment III, 3 h, 10 μCi/ml, spec. act. 25 Ci/mmole. GM03248 (RO), GM03248 used in experiments II and III obtained from the Camden Cell Bank repository; GM03248 (BE), cell batch kindly provided by Dr. Robbins (Bethesda, MD) and used by Moshell et al. (1983); XP6BE (RO), Rotterdam cell line, kept in our laboratory for several years; XP6BE (BE), cell batch kindly provided by Dr. J. Robbins (Bethesda, MD) and used by Moshell et al. (1983).


A
B

ences of the XP cell strains used, see Table 1). To unambiguously identify each type of dikaryon (homodikaryons of the 2 cell strains used and heterodikaryons) fusion partners were labeled with beads of different size prior to cell hybridization. The results of grain counting after the UDS assay are summarized in Table 2 (experiment I). Micrographs showing the UDS of XPCS2-I/XP1BR and XPCS2-I/XP25RO heterokaryons are presented in Fig. 1.

As expected, no complementation was found between the 2 XP-D strains. The fusion of XPCS2-I with XP25RO (XP-A, see Fig. 1B) shows that XPCS2-I fibroblasts can be complemented to the wild-type UDS level (Table 2). However, no complementation was observed between XPCS2-I and XP1BR (XP-D, see Fig. 1A and Table 2).

To exclude the possibility that our XPCS2-I strain is in fact mixed up with an XP-D strain, this cell strain was compared with 2 other cultures of the XP-H patient by DNA fingerprint analysis which produces a DNA pattern that is completely individual-specific (Jeffreys et al., 1985). The 2 other cultures were XPCS2-II, a fibroblast culture obtained in 1982 and stored in liquid nitrogen since its arrival in our laboratory, and the reference XP-H cell strain from the Camden Cell Bank, GM03248, which was also utilized by Moshell and coworkers. DNA isolated from these cells and an unrelated control (MK) was digested, separated and blotted to a filter as described in Materials and methods. The autoradiogram after hybridization with a labeled minisatellite probe (Jeffreys et al., 1985) is shown in Fig. 2. All 3 XPCS2 strains have exactly the same pattern of bands, which is different from that of an unrelated individual. This rules out mixing up of cell strains as an explanation for the absence

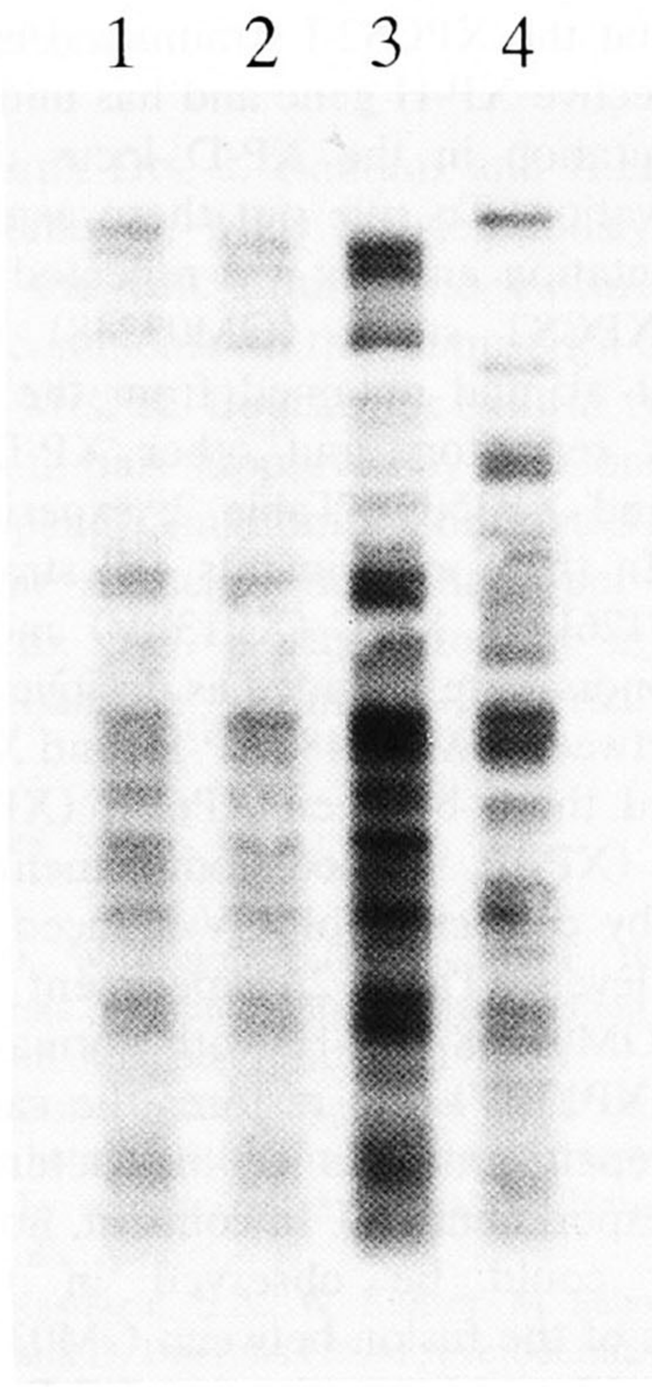


Fig. 2. DNA fingerprint analysis of various XPCS2 strains. DNAs (10 μg) were digested with restriction endonuclease *Hin*fI, separated on a 0.8% agarose gel, blotted to a nitrocellulose filter and hybridized with a minisatellite probe 33.15. Lane 1, reference XP-H cell line from Camden Cell Bank GM03248; lane 2, XP-H cell line from frozen stock Rotterdam (XPCS2-II); lane 3, XP-H cells used for fusion experiment I (XPCS2-I); lane 4, non-related control DNA (MK).

of complementation between XPCS2-I and an XP-D line.

A theoretical, but unlikely explanation for the lack of complementation is that the XP1BR (XP-D) strain is a double mutant, affected in both the XP-D and the XP-H locus. Alternatively, it is

Fig. 1. Micrograph of XP heterokaryons after UV-induced UDS and autoradiography. (A) Fusion between XPCS2-I (XP-H) and XP1BR (XP-D) primary fibroblasts. The XP-H cells were labeled with 2-μm latex beads and the XP-D strain with 0.8-μm beads. The heterokaryon (arrow) is identified by the presence of both types of beads. Two days after fusion the cells were exposed to 15 J/m^2 UV light, incubated in the presence of tritiated thymidine, fixed and processed for autoradiography. The number of grains above nuclei is a measure of the level of UDS. The XPCS2-I monokaryon with the density-labeled nucleus was in S-phase during the incubation in ^{3}H-TdR. No increased level of UDS is observed above the 2 nuclei of the XP-D/XP-H heterokaryon compared to the non-fused monokaryons. (B) Fusion between XPCS2-I (XP-H) and XP25RO (XP-A) primary fibroblasts. The XP-H cells were labeled with 2-μm latex beads and the XP-A strain with 0.8-μm beads. The heterokaryon (arrow) is identified by the presence of both types of beads. The procedure is the same as described in (A). The XP-A/XP-H heterokaryon shows a normal level of UDS. Note that the residual level of UDS in the XP-A fibroblasts is significantly lower than that of the XP-H monokaryon.

possible that the XPCS2-I strain used by us harbors a defective XP-H gene and has undergone a second mutation in the XP-D locus during in vitro cultivation. To rule out these explanations complementation analysis was repeated with the original XPCS2 strain (GM03248) used by Moshell et al. and obtained from the Camden Cell Bank repository and other XP-D strains (XP6BE and XP3NE) (Table 2, experiments II and III). In these experiments cell strains from XP-F (XP126LO), XP-C (XP13PV) and normal (C1PV) donors were included as positive controls. Fusions between GM03248 (XP-H) and XP126LO (XP-F) and those between XP6BE (XP-D) and XP126LO (XP-F), yielded complementation as indicated by correction of UV-induced UDS to wild-type levels (Table 2, experiment II). The fusion of GM03248 (XP-H) with normal (C1PV) or XP-C (XP13PV) also restores the capacity to perform repair synthesis in the heterokaryons (Table 2, experiment III). In contrast, no complementation could be observed in the heterokaryons of the fusion between GM03248 (XP-H) and the 2 representatives of XP-D (XP6BE and XP3NE). In a final attempt to imitate as much as possible the experimental conditions employed by Moshell and coworkers (1983), we performed complementation using culture medium made by the same manufacturer, and according to the specifications used by Moshell et al., serum ordered from the same manufacturer as used by Moshell et al. (not from the same batch) and the same cell batches that were utilized in the original fusions by Moshell et al. (1983) (kindly provided by Dr. Robbins, Bethesda, MD). As shown in Table 2, experiment IV, again no evidence for complementation could be obtained, ruling out the unlikely possibility that differences in culture conditions underlie the discrepancy. From these data we have to conclude that XPCS2 (GM03248), originally assigned to XP complementation group H, cannot complement XP group D in agreement with observations made by Johnson et al. (1989).

Discussion

Confusion about the status of XP group H in the literature (Robbins, 1989; Johnson, 1989) prompted us to carefully reinvestigate the complementation analysis between XP groups H and D. Complementation data are important with respect to the number of genes implicated in human excision-repair and in XP. The high number of excision-defective XP complementation groups identified thus far makes complementation analysis not only very laborious, but also complicated and susceptible to errors. Here we provide evidence that fibroblasts of patient XPCS2 (GM-03248), previously assigned by Moshell et al. (1983) to the new complementation group H, do not complement cells from XP complementation group D, as determined by the classical parameter for XP complementation analysis; i.e., the assay for UV-induced UDS. This finding confirms the observations of Johnson et al. (1989) made in the course of characterizing a HeLa-XP-D hybrid line who could not demonstrate recovery of biological activity after UV or improved incision in permanent XP-D/XP-H hybrid cells. On the other hand, it disagrees with the reports of Moshell et al. (1983), where the data on XPCS2/XP-D complementation were not shown, and with the data of Fujiwara and Satoh (1985) and Ichihashi et al. (1988) who found complementation between GM03248 and one of their reference XP-D strains. In our experiments (as well as in those of Fujiwara and Satoh (1985) and Ichihashi et al. (1988)) latex beads were used to unambiguously identify heterokaryons and determine each type of homokaryon (see Fig. 1). Furthermore, trivial explanations, such as mixing up of cell strains, contamination of XP lines with each other, or differences in culture conditions have been ruled out. The unlikely theoretical possibility of a double mutation in either XPCS2 or XP1BR(D) has been dismissed as well. Since these controls have been included in this study we believe that we have convincingly demonstrated the inability of XPCS2 cells to complement XP-D and conclude that XPCS2 is a member of XP group D. This reduces the number of excision-deficient XP complementation groups to 7. The identity of XP-D and XP-H fits well with our previous observations on the effect of injected yeast photoreactivating enzyme (PRE) on the residual UDS of XP-D and XP-H fibroblasts (Zwetsloot et al., 1986). In both cell strains, which now appear to belong to the same complementa-

tion group, photoreactivation of dimers by the injected PRE did not result in a detectable decrease in residual UDS when measured 1–2 h after UV irradiation and illumination with photoreactivating light. In XP-C and formerly XP-I (Bootsma et al., 1989) as well as in XP-F cells photoreactivation of dimers did result in a decrease of residual UDS as was found with wild-type cells. Incorporation of patient XPCS2 into group D extends the clinical heterogeneity within this group, which was already associated with the rare hereditary disorder trichothiodystrophy (TTD) (Stefanini et al., 1986). The molecular basis of the intriguing combined occurrence of XP plus CS and XP plus TTD within this complementation group will have to await cloning of the defect gene(s) in these disorders. In the other case of the combination of XP and CS (XP11BE, XP complementation group B), the molecular defect was recently elucidated (Weeda et al., 1990): a C → A transversion in the splice acceptor of the last intron of the human excision-repair gene *ERCC-3* caused a 4-bp insertion in the mature mRNA and a C-terminal frameshift in the encoded protein of the single XPCS-1 patient. On the basis of the deduced amino acid sequence the *ERCC-3* gene product is postulated to have a DNA-unwinding function. It will be of interest to determine which mutations underlie the clinical heterogeneity within the XP-D group.

Note added in proof

In a recent meeting (Noordwijkerhout, The Netherlands, 14–19 April 1991), C.A. Weber and L.H. Thompson (Livermore) presented evidence that the excision-repair gene *ERCC-2* upon transfection specifically corrects the UV sensitivity of XP group D cells as well as XP-H cells in a transient differential cytotoxicity assay. This implies that a single gene is involved, which is in agreement with our findings. Also J.H. Robbins (Bethesda) informed us recently that he now concludes from current and past research that the claim of the existence of XP complementation group H should be withdrawn and that strain GM 3248 should be reassigned to XP complementation group D (J.H. Robbins, personal communication).

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