

UV Stimulation of DNA-Mediated Transformation of Human Cells

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Irradiation of dominant marker DNA with UV light (150 to 1,000 J/m²) was found to stimulate the transformation of human cells by this marker from two- to more than fourfold. This phenomenon is also displayed by xeroderma pigmentosum cells (complementation groups A and F), which are deficient in the excision repair of UV-induced pyrimidine dimers in the DNA. Also, exposure to UV of the transfected (xeroderma pigmentosum) cells enhanced the transfection efficiency. Removal of the pyrimidine dimers from the DNA by photoreactivating enzyme before transfection completely abolished the stimulatory effect, indicating that dimer lesions are mainly responsible for the observed enhancement. A similar stimulation of the transformation efficiency is exerted by 2-acetoxy-2-acetylaminofluorene modification of the DNA. No stimulation was found after damaging vector DNA by treatment with DNase or γ rays. These findings suggest that lesions which are targets for the excision repair pathway induce the increase in transformation frequency. The stimulation was found to be independent of sequence homology between the irradiated DNA and the host chromosomal DNA. Therefore, the increase of the transformation frequency is not caused by a mechanism inducing homologous recombination between these two DNAs. UV treatment of DNA before transfection did not have a significant effect on the amount of DNA integrated into the xeroderma pigmentosum genome.

UV light induces a variety of lesions into DNA, such as pyrimidine dimers (the main product), thymidine glycols, 6-4 photoproducts, and DNA protein cross-links. Deformation of the DNA double-helix structure by these lesions disturbs essential processes, such as replication and transcription, and ultimately can result in mutagenesis and lethality. The major repair system operating on many of these lesions is the excision repair pathway (see references 19, 22, and 29 for reviews). Irradiation of prokaryotic and eukaryotic cells with UV light and in some cases transfection with DNA exposed to UV light induces a variety of processes, such as stimulation of recombination (13, 17, 21, 23), enhancement of survival of UV-damaged viruses (host cell reactivation; 6, 8, 9, 37) or bacteriophages (Weigle reactivation; 46), enhanced capacity to repair potentially lethal damage (45), and increased mutagenesis (7-9, 12, 37). In *Escherichia coli* many of these phenomena are part of the pleiotropic SOS response (reviewed in reference 30). In eukaryotes several of these features are expressed concomitantly, and it is likely that they are at least in part related to each other. However, the nature of the inducing signal(s) is still unknown. Here we report that UV irradiation has a stimulatory effect on the genetic transformation of human cells. This phenomenon was encountered in the course of experiments aimed at the cloning of a human DNA repair gene complementing the excision repair defect of xeroderma pigmentosum (XP) cells. The rationale of this approach was to rescue a UV-damaged dominant marker when cotransfected with the XP-correcting wild-type repair gene present in carrier DNA. It appeared that UV irradiation of the dominant marker did not exercise a deleterious but rather a stimulatory effect on the transformation frequency of XP and normal cells. This finding, which confirms recently reported observations of Spivak et al. (41), is studied in more detail in the present paper. Evidence is presented that pyrimidine dimers are the responsible UV lesions. A similar effect is exerted by modification of DNA by *n*-acetyl-aminofluorene (AAF). This carcinogen preferentially interacts with the C-8

position of guanine, resulting in guanine-AAF (27, 33), which is like pyrimidine dimers removed by excision repair (16). Finally, we demonstrate that homologous recombination between transfected DNA and the genome of the recipient cells is not responsible for UV stimulation of the transformation.

MATERIALS AND METHODS

Cell lines and culture conditions. XP12ROSV40 (10) and XP2YO(SV) (49) are simian virus 40 (SV40)-transformed excision repair-deficient XP fibroblasts of complementation groups A and F, respectively. CHO12RO is an excision repair deficient-Chinese hamster ovary (CHO) cell line (43). SV40wtA, an SV40-transformed human fibroblast, a wild-type CHO cell line, and HeLa-S3 cells were used as repair-proficient cell lines.

The cell lines were grown in F10 medium (without thymidine) and Dulbecco minimal essential medium (1:1) supplemented with antibiotics and 3% fetal and 7% newborn calf serum.

Isolation of DNA. High-molecular-weight DNA of HeLa-S3 cells was isolated as described (24). Plasmid DNA was isolated by using the alkaline lysis procedure (5).

Induction of DNA damage. UV irradiation of plasmid DNA (and, where specified, HeLa DNA) was carried out in a maximum volume of 250 μ l on a watch-glass by using a UV source at a fluence of 0.6 J/m². For high UV doses the distance to the lamp was decreased. The DNA concentration during irradiation ranged from 0.1 to 0.4 mg/ml. UV-irradiated DNA (and nonirradiated control DNA) was ethanol precipitated before use for transfection or analysis on alkaline gels.

X irradiation of plasmid DNA was done at a DNA concentration of 4 mg/ml in a closed Eppendorf tube by using a Philips X-ray machine (10 mA; 175 rads/min).

Modification of plasmid DNA with AAF was performed as described by Landegent et al. (28).

Transfection and selection. The transfection protocol was as described (18, 48), with some modifications. One day before transfection 10 petri dishes were seeded with 2×10^5

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to 5×10^5 cells per dish. DNA (2 μ g of plasmid DNA and 8 to 10 μ g of HeLa carrier DNA) was added to the cells as a calcium phosphate precipitate. After exposure to the DNA (5 h for human and 12 h for CHO cells) cells were subjected to a dimethyl sulfoxide shock (10%; 30 min) and subsequently cultured in nonselective medium for 48 h, after which selection was started. Usually 10 to 14 days later colonies were fixed and stained. Only colonies of more than 20 cells were counted. Unless indicated otherwise four plates were used to determine the average number of colonies per plate (\pm standard error of the mean [SEM]). Dominant markers harboring the Tn5 neomycin resistance gene were pSV₂neo (40) and the cosmid vectors pTCF (20), pMCS (20), and pMCS-Alu (see also Fig. 4). pMCS is identical to pTCF except for the fact that it carries an SV40 *ori* region in front of the thymidine kinase (TK) promoter. pMCS-alu contains one copy of a human Alu repeat cloned in the unique *Bam*HI site of pMCS. The dominant marker pSV₃gptH (47) contains the *eco-gpt* gene that gives resistance to mycophenolic acid.

Cells transfected with pSV₃gptH were selected in mycophenolic acid medium as specified (47). For selection of cells transfected with dominant markers carrying the Tn5 neomycin resistance gene, the normal medium was supplemented with G-418 (300 μ g/ml; Schering Corp.). The selection medium was refreshed every 2 to 3 days.

UV irradiation of cells. To determine the transformation frequency after UV exposure of the recipient cells, cells were irradiated immediately after the dimethyl sulfoxide shock by using a UV lamp at a fluence of 0.3 J/m² per s. Parallel to the transfection experiment the survival at the same UV dose was determined. After irradiation the cells were trypsinized, and 2,000 cells were seeded onto 10-cm petri dishes. After 11 days the number of clones was determined and compared with that of nonirradiated control cells. The cloning efficiency was ~25%.

Enzyme treatments of DNA. (i) Restriction endonucleases. Digestions of DNA with restriction enzymes and DNase I were done as recommended by the suppliers (Boehringer; New England Biolabs).

(ii) T4 endonuclease V. Plasmid DNA (UV irradiated) was incubated with purified T4 endonuclease (a generous gift of

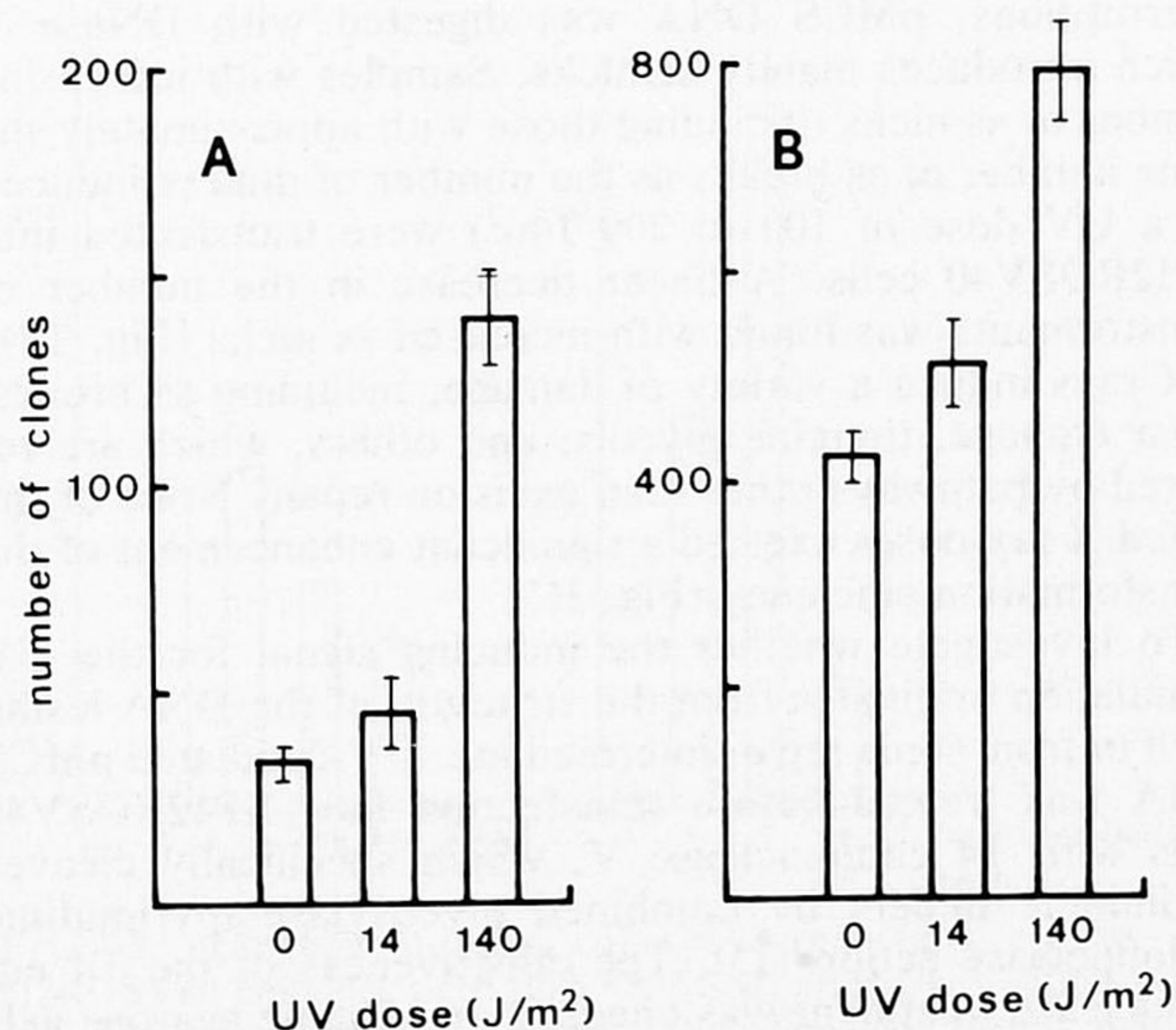


FIG. 1. Average number of G418-resistant clones per petri dish (\pm SEM) after transfection of UV-irradiated pMCS DNA to XP12ROSV40 (A) and SV40wtA (B) cells.

TABLE 1. Effect of UV-irradiation of dominant marker genes on transformation frequency of various mammalian cells^a

Transfected cell line	Dominant marker	UV dose (J/m ²)	No. of transformants (\pm SEM)	Stimulation (fold)
XP12ROSV40	pMCS	0	240 \pm 16	2.6
		200	627 \pm 36 ^b	
XP12ROSV40	pSV ₂ neo	0	13 \pm 5	11
		200	142 \pm 25	
XP2YO (SV)	pMCS	0	15 \pm 2	3.7
		200	54 \pm 4	
SV40wtA	pMCS	0	107 \pm 17	1.7
		200	177 \pm 15	
HeLa-S3	pSV ₃ gptH	0	122 \pm 21	2
		200	243 \pm 15	
CHO12RO	pSV ₃ gptH	0	596 \pm 127 ^b	1.1
		140	656 \pm 72 ^b	
		700	678 \pm 56 ^b	
CHO	pSV ₃ gptH	0	802 \pm 121 ^b	1.1
		140	878 \pm 136 ^b	
		7,000	406 \pm 38 ^b	

^a See the text for experimental procedures.

^b Individual clones are difficult to score at colony densities higher than ca. 500, leading to underestimation of the actual number of transformants (and probably also of the UV stimulation).

A. A. van Zeeland; State University, Leiden, The Netherlands) for 30 min at 37°C in 10 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA–100 mM NaCl–0.3 mg nuclease-free bovine serum albumin (Biolabs) per ml.

PRE. Treatment of plasmid DNA (UV irradiated) with purified photoreactivating enzyme (PRE) from *Anacystis nidulans* (generously provided by A. P. M. Eker, University of Technology, Delft, The Netherlands) was performed in 10 mM potassium phosphate buffer (pH 7.0)–10 mM NaCl. Because of the lability of PRE at 37°C, fresh enzyme was added after 0, 20, and 50 min. The samples in glass tubes were illuminated with photoreactivating light (TLADK 30W/03 lamp; Philips) at ca. a 10-cm distance throughout the entire incubation period (1 h at 37°C).

Gel electrophoresis of DNA. DNA fragments were separated by electrophoresis in 0.5% horizontal agarose gels (31). For qualitative determination of the single-stranded (ss) breaks, DNA was analyzed on alkaline agarose gels as described (31).

RESULTS

Effect of UV irradiation of vector DNA on the transformation frequency of mammalian cells. The dominant marker pMCS was irradiated with various UV doses and transfected to XP12ROSV40 cells and repair-competent SV40wtA cells. Upon UV irradiation a significant increase in the number of transformants was obtained with both cells (Fig. 1). To investigate the generality of this phenomenon, transfection experiments were carried out with different dominant marker genes and a number of repair-proficient and repair-deficient recipient cells. The results are summarized in Table 1.

A significant increase was found with the vectors pMCS and pSV₂neo, both of which contain the neomycin resistance gene, as well as with vector pSV₃gptH, which harbors the *E. coli gpt* gene that renders the cell resistant to mycophenolic acid medium. This suggests that stimulation of the transformation frequency is not dependent on the type of vector or dominant marker used. Furthermore, the addition or omission of unirradiated carrier DNA during transfection did not influence the observed effect (data not shown).

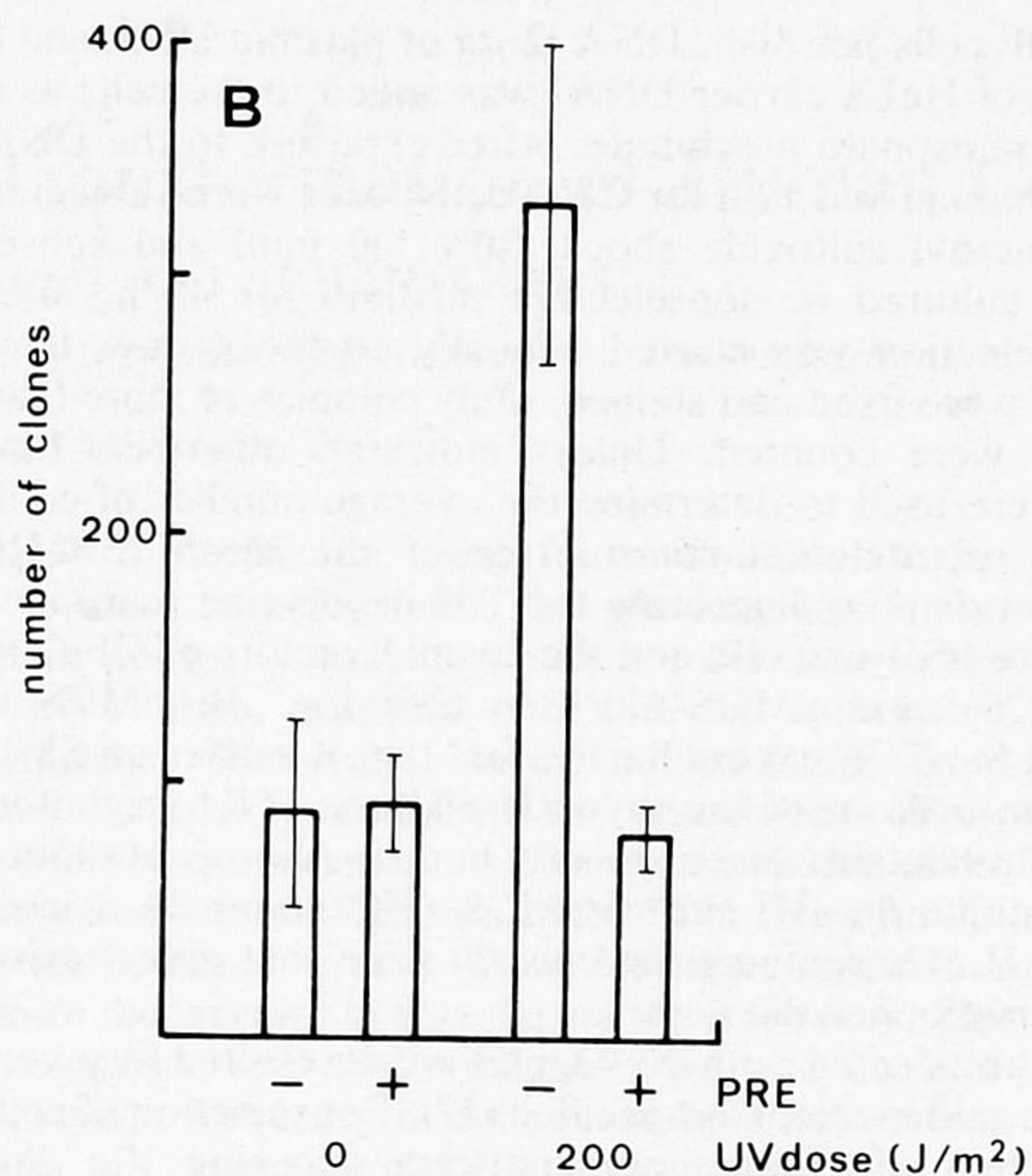
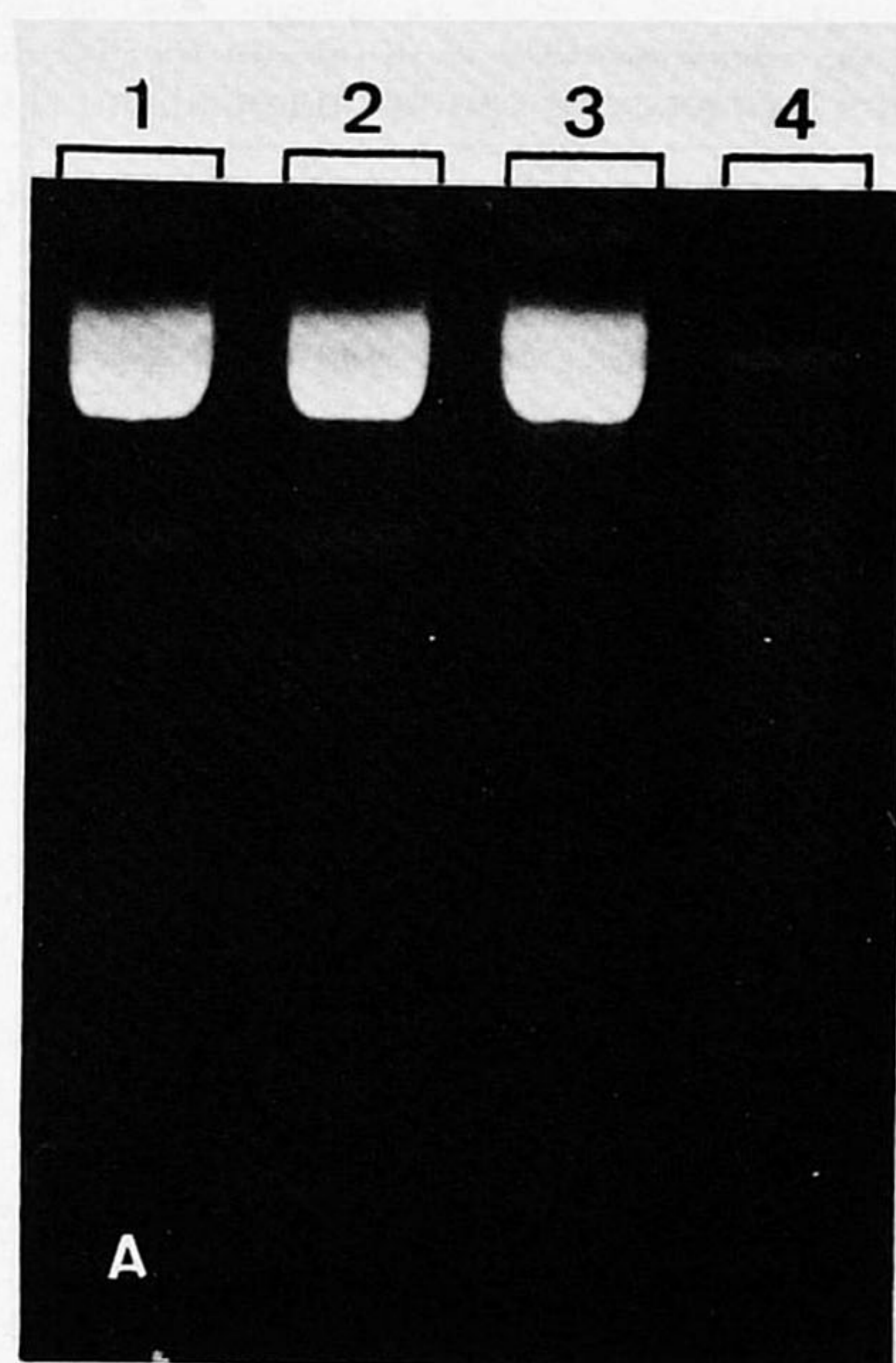


FIG. 2. (A) Alkaline agarose gel (0.6%) showing the effect of the PRE of *A. nidulans* on UV-treated pMCS DNA. Before electrophoresis 4 μ g of DNA was incubated with T4 endonuclease to generate dimer-dependent ss breaks. Lanes: 1, 0 J/m² with PRE; 2, 0 J/m² without PRE; 3, 200 J/m² with PRE; 4, 200 J/m² without PRE. (B) Average number of G418-resistant clones per petri dish (\pm SEM) after transfection of XP12ROSV40 cells with PRE-treated, UV-irradiated pMCS-DNA.

XP cells showed stimulation in the same order of magnitude as repair-competent human cells. In contrast no stimulation was found with two CHO cell lines. It should be noted, however, that these cells already have a very high transformation frequency. These results indicate that UV stimulation of the transformation is a reproducible phenomenon, not influenced by the excision repair characteristics of the recipient cells.

Although in all experiments UV irradiation consistently stimulated transformation, the degree of stimulation varied between individual experiments with the same cell lines and vectors. In most experiments, however, an increase by a factor of 2 to 4 was found.

The maximal stimulatory effect was observed with UV doses between 150 and 1,000 J/m². At higher doses transformation efficiencies decreased to values below that of the unirradiated control (but even at doses of 7,000 J/m² transformants were observed [data not shown]; see also Table 1).

Effect of photoreactivation of UV-irradiated vector DNA on stimulation of the transformation frequency. Since UV light induces a number of different lesions in DNA it was of interest to establish which particular UV lesion in the dominant vector was responsible for the stimulatory effect. Before transfection, UV-irradiated plasmid DNA was treated in vitro with purified PRE from the cyanobacterium *A. nidulans*. This enzyme specifically monomerizes pyrimidine dimers. The activity of PRE was tested by digesting PRE- and non-PRE-treated UV-irradiated DNA with T4 endonuclease V (which introduces an ss break at the position of a pyrimidine dimer) and separating the fragments by alkaline agarose gelelectrophoresis. The UV-irradiated DNA in the PRE-treated sample is not cleaved by T4 endonuclease, in contrast to non-PRE-treated DNA, indicating that PRE treatment resulted in the removal of pyrimidine dimers (Fig. 2A). Figure 2B demonstrates that as a result of PRE treatment UV stimulation of the transformation efficiency is abolished, which suggests that pyrimidine dimers are responsible for the stimulatory effect.

Effect of other DNA-damaging treatments on the transformation frequency of DNA. To investigate whether stimulation of the transformation frequency is elicited specifically by UV damage, vector DNA was subjected to other DNA-damaging treatments (such as AAF modification, X rays, and DNase) before transfection. pMCS DNA was treated with AAF to a modification level of 0.5% (i.e., 2% of all guanine residues), which is approximately equivalent to the number of pyrimidine dimers in the DNA introduced by a UV dose of 200 J/m². As shown by Fig. 3A, 0.5% AAF modification of the DNA induces a 2.1-fold stimulation of the transformation frequency in XP12ROSV40 cells.

In the course of the excision repair process, ss breaks or gaps are introduced at or near to dimers. To investigate whether the stimulatory effect originates from such ss DNA interruptions, pMCS DNA was digested with DNase I, which introduces mainly ss nicks. Samples with increasing number of ss nicks (including those with approximately the same number of ss breaks as the number of dimers induced by a UV dose of 100 to 200 J/m²) were transfected into XP12ROSV40 cells. A linear decrease in the number of transformants was found with increased ss nicks (Fig. 3B).

X rays induce a variety of damage, including ss breaks, sugar damage, thymine glycols, and others, which are repaired by pathways other than excision repair. None of the tested X ray doses exerted a significant enhancement of the transformation efficiency (Fig. 3C).

To investigate whether the inducing signal for the UV stimulation originates from the structure of the DNA lesion itself or from some repair intermediate, UV-irradiated pMCS DNA was treated before transfection into XP12ROSV40 cells with T4 endonuclease V, which specifically cleaves pyrimidine dimers by combined glycosylase-apyrimidinic endonuclease action (15). The effectiveness of the T4 endonuclease treatment was checked on alkaline agarose gels (data not shown). Pretreatment of the UV-damaged DNA with T4 endonuclease V completely abolished stimulation of the transformation efficiency (Fig. 3D). In fact, compared

with nonirradiated pMCS, a drastic decrease in the number of transformants was even observed, which resembled the effect of DNase.

Effect of UV irradiation of recipient cells on the transformation frequency of dominant marker genes. To investigate whether UV irradiation of the host cell also induces a stimulatory effect on the transformation efficiency, XP12ROSV40 and repair-proficient SV40-transformed human fibroblasts were transfected with pMCS DNA and UV irradiated immediately after transfection (0.5 J/m^2 for XP cells; 0.5 and 5 J/m^2 for SV40wtA cells). Irradiation of cells did not result in stimulation comparable to that found after UV irradiation of vector DNA (Table 2). However, taking into account the survival of XP and wild-type cells at the UV doses tested, considerable stimulation was found, particularly in the case of XP cells.

Role of homologous recombination between exogenous and host cellular DNA in UV-stimulated transformation frequency. To investigate whether DNA sequence homology between the UV-damaged dominant vector and genomic

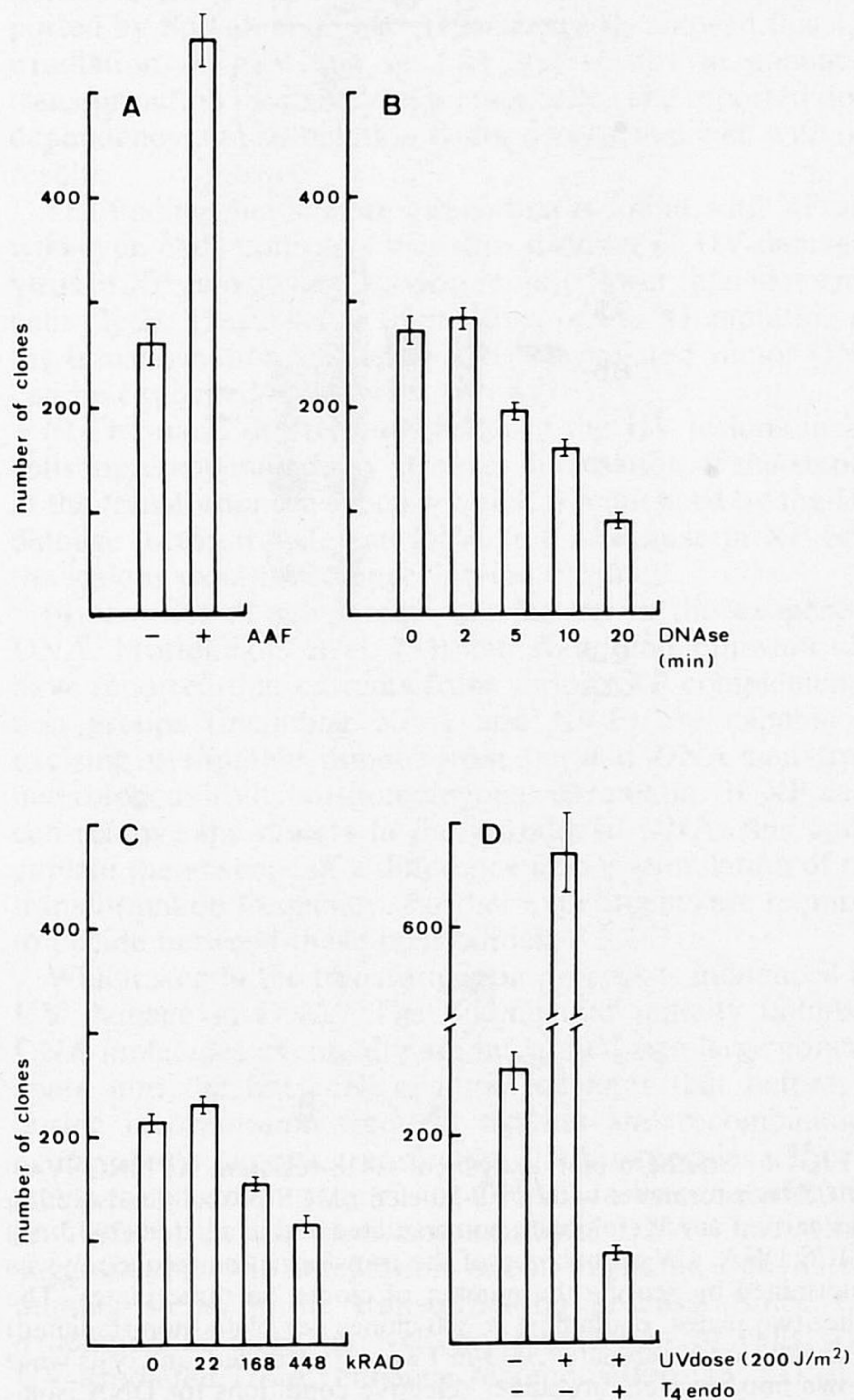


FIG. 3. Average number of G418-resistant clones of XP12ROSV40 cells per petri dish (\pm SEM) after transfection with pMCS DNA treated with different DNA-damaging agents before transfection. (A) AAF modification (0.5%); (B) partial DNase digestion (incubation times indicated); (C) X irradiation; (D) UV plus T4 endonuclease incubation.

TABLE 2. Effect of UV irradiation of recipient cells on the transformation frequency of pMCS

Cell line	UV dose (J/m^2)	No. of clones (\pm SEM) ^a
XP12ROSV40	0	341 ± 20
	0.5	404 ± 30 (2,020)
SV40wtA	0	703 ± 28
	0.5	897 ± 65
	5.0	863 ± 42 (1,233)

^a Values in parentheses represent the number of clones after correction for cell survival. At a dose of 0.5 J/m^2 XP12ROSV40 cells displayed a survival rate of ca. 20%, as determined in a parallel experiment. SV40wtA cells had a survival rate of 70 to 80% after UV irradiation with 5 J/m^2 .

DNA of recipient cells plays a role in the enhancement of transformation, three types of experiments were carried out. Two pMCS derivatives, one without SV40 (*ori*⁻) sequences (pTCF) and one containing a human Alu sequence (pMCS-alu) (see Fig. 4, upper part), were UV irradiated (200 J/m^2) and transfected to XP12ROSV40 cells. The results (Fig. 4, lower part) demonstrate that irradiation of the vector DNA in all cases induces approximately the same relative stimulation. UV irradiation of pMCS-alu, which possesses homology with ca. 4×10^5 Alu copies in the human genome, gives the same relative increase in transformation efficiency as UV irradiation of vector pMCS, which has only SV40 sequence homology, and vector pTCF, which has no homology at all with the XP12ROSV40 genome. Furthermore, this experi-

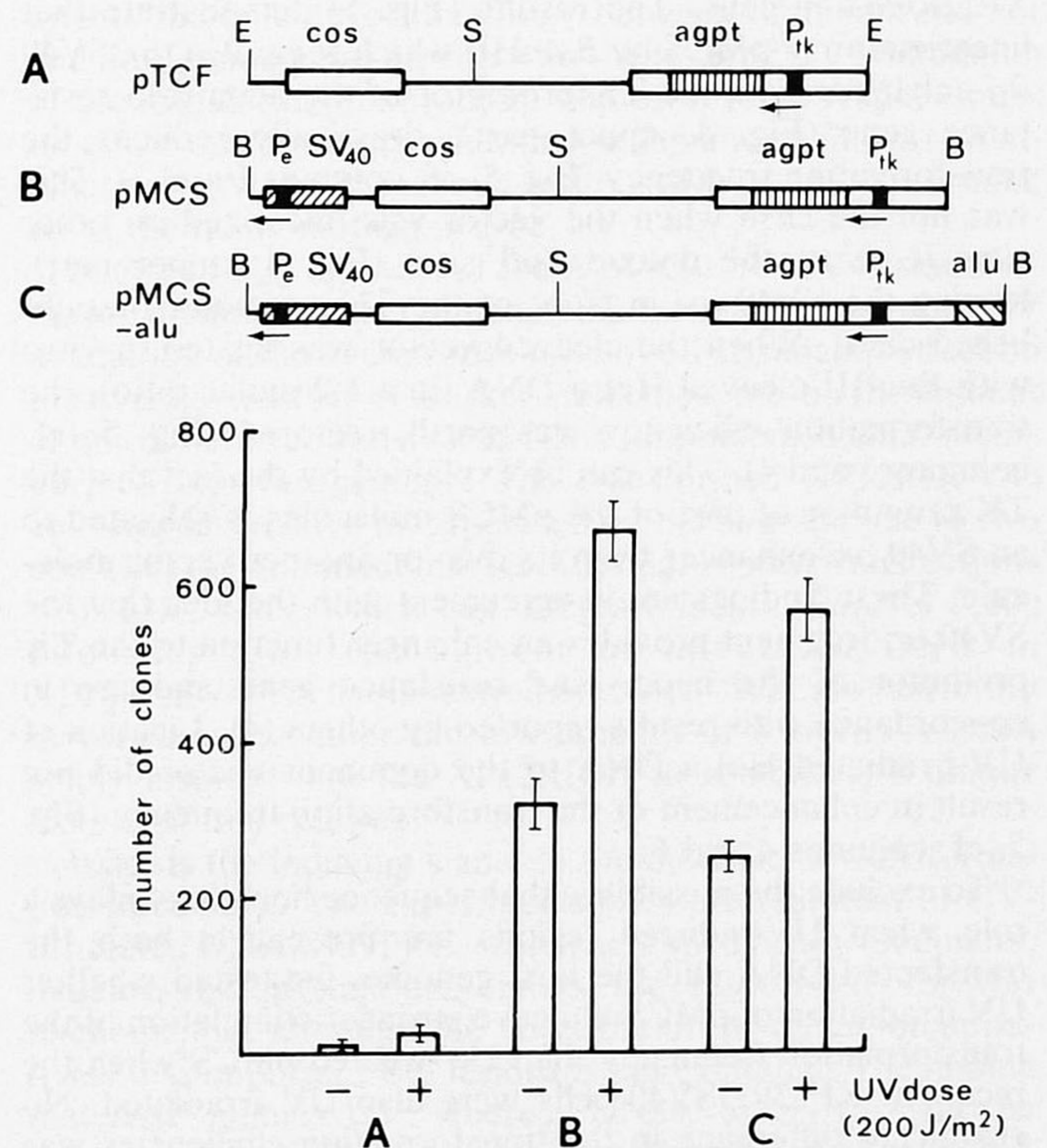


FIG. 4. UV stimulation of the transformation of XP12ROSV40 cells with different UV-irradiated plasmids harboring the G418 resistance gene. *P_e* and *P_{tk}* represent early promoter from the SV40 *ori*-enhancer fragment and herpes TK promoter, respectively. *agpt*, Aminoglycosylphosphoribosyltransferase gene, encoding resistance against G418; *E*, *EcoRI*; *B*, *BamHI*; *S*, *Sall*; *cos*, *cos* sequence of bacteriophage lambda. Transfection was with pTCF (A), pMCS (B), and pMCS-alu (C).

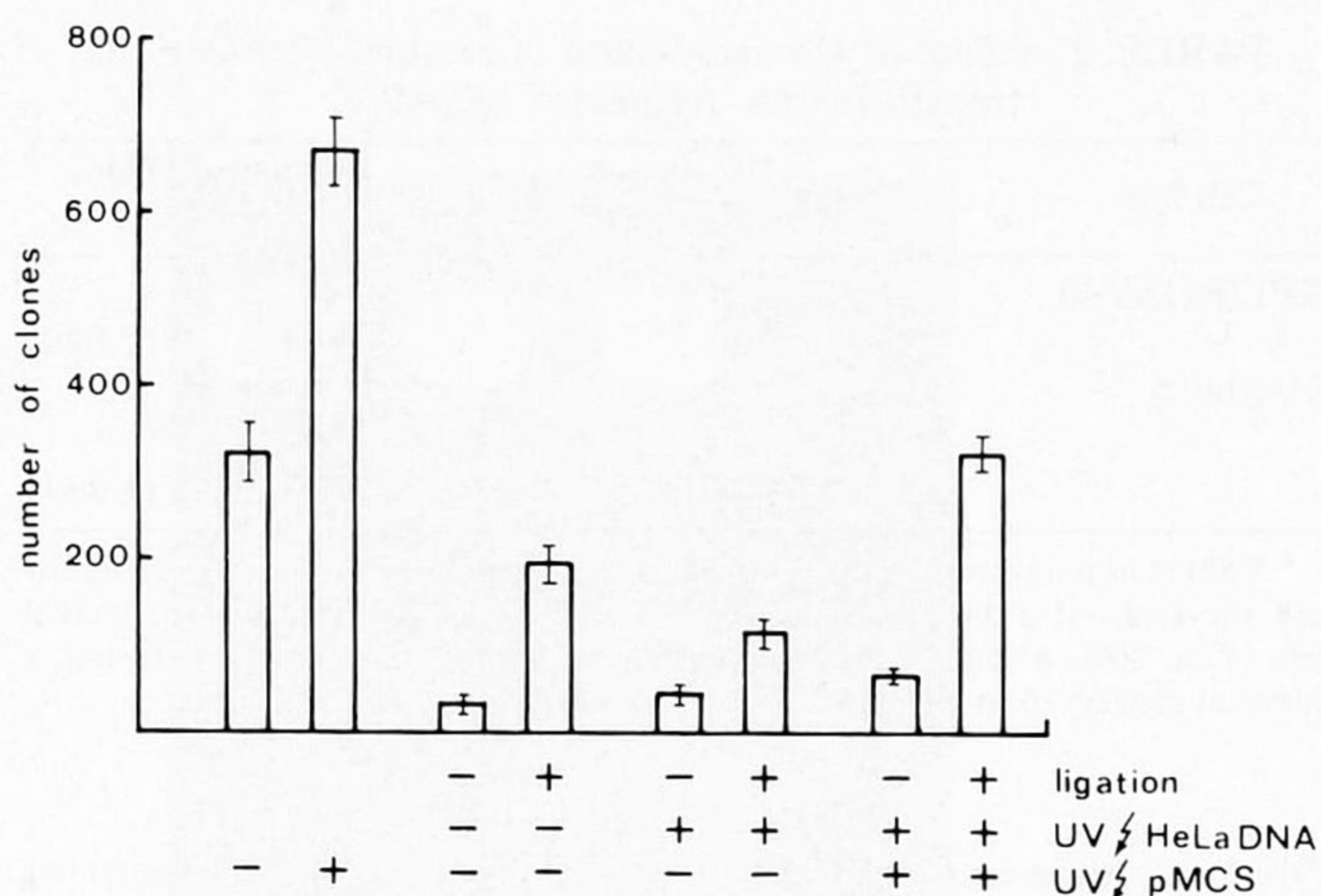


FIG. 5. G418-resistant clones after transfection of XP12ROSV40 cells with *Bam*HI-digested pMCS DNA ligated to *Bam*HI-cleaved HeLa DNA. When indicated, DNAs were irradiated with a UV dose of 200 J/m². The first two lanes represent control experiments with undigested pMCS DNA.

ment shows that potential extra-chromosomal replication of vector pMCS, due to the SV40 origin, is not involved in the process that leads to stimulation of the transformation frequency. It is evident that deletion of the SV40 *ori*-region reduces the transformation frequency more than 30-fold.

In the second experiment UV-irradiated and nonirradiated human DNA fragments (obtained after digestion of HeLa DNA with restriction endonuclease *Bam*HI) were ligated in vitro to *Bam*HI-linearized pMCS and transfected into XP12ROSV40 cells. The results (Fig. 5) demonstrate that linearization of pMCS by *Bam*HI, which separates the SV40 *ori*-enhancer from the TK promoter of the neomycin resistance gene (Fig. 4, upper part), drastically reduces the transformation frequency (Fig. 5, cf. columns 1 and 3). This was not the case when the vector was linearized on other sites (e.g., at the unique *Sal*I site) (Fig. 4, upper part), leaving the SV40 *ori* in front of the TK promoter (unpublished data). When the cleaved vector was ligated in vitro with *Bam*HI-cleaved HeLa DNA (in a 1:2 molar ratio), the transformation efficiency was partly restored (Fig. 5, cf. columns 3 and 4). This can be explained by the fact that the TK promoter of part of the pMCS molecules is religated to an SV40 *ori*-enhancer from its own or another vector molecule. These findings are in agreement with the idea that the SV40 *ori* fragment provides an enhancer function to the TK promoter of the neomycin resistance gene and are in concordance with results reported by others (4). Ligation of UV-irradiated HeLa DNA to the dominant vector did not result in enhancement of the transformation frequency (Fig. 5, cf. columns 4 and 6).

To exclude the possibility that sequence homology plays a role when UV-induced lesions are present in both the transfected DNA and the host genome, we tested whether UV irradiation of pMCS-*alu* gave stronger stimulation of the transformation frequency than UV-treated pMCS when the recipient XP12ROSV40 cells were also UV irradiated. No significant difference in the transformation efficiencies was observed between the two vectors (data not shown). The data presented above rule out the possibility that stimulation of the transformation frequency is caused by enhanced homologous recombination between (damaged) vector and host DNA.

Effect of UV irradiation on integration of vector DNA. It is of interest to know whether UV irradiation has an effect on

the number of vector copies or on the amount of unirradiated cotransferred carrier DNA that integrate in the genome of the recipient cell. Southern blot analysis was done on XP12ROSV40 cells transfected with (UV-irradiated) pMCS. After transfection and selection, DNA was extracted from the total transformed cell population, originating from petri dishes with 300 to 600 independent clones. This DNA was digested with restriction endonucleases, and the fragments were size fractionated by agarose gel electrophoresis. Southern blots of the gel were hybridized with ³²P-labeled pMCS DNA. The autoradiogram of the hybridized filter shown in Fig. 6 demonstrates that there is no notable difference in the intensity of vector fragments between the irradiated and nonirradiated DNA samples. Since the DNA is derived from 300 to 600 independent clones, the relative intensity of the internal vector fragments gives the average number of integrated copies per cell. From the intensity of coelectropho-

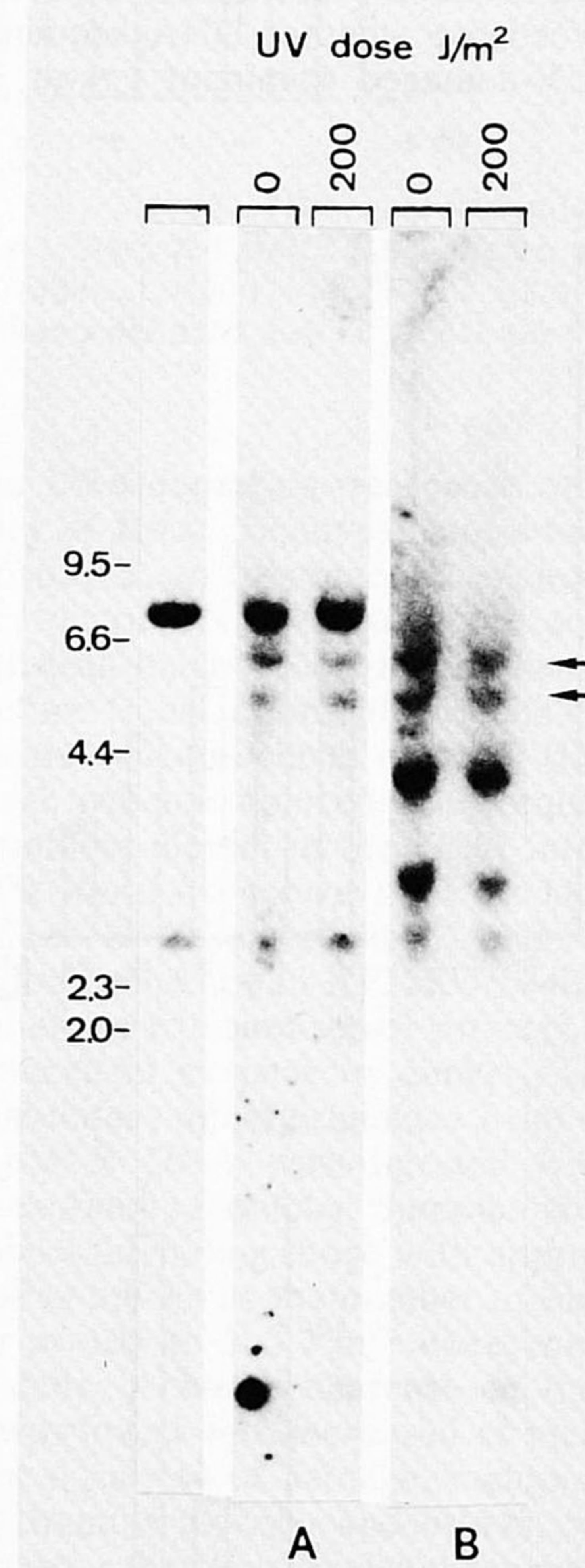


FIG. 6. Southern blot analysis of G418-resistant XP12ROSV40-pMCS transformants with a ³²P-labeled pMCS probe. Transfection was carried out fivefold with nonirradiated and irradiated (200 J/m²) pMCS DNA. UV stimulation of the transformation frequency was determined by scoring the number of clones on three plates. The other two plates, containing \pm 300 clones per plate (nonirradiated) or \pm 600 clones per plate (200 J/m²) were trypsinized, and cells were grown into mass culture under selective conditions for DNA isolation. DNA (15 μ g) was digested with *Eco*RI (A) or *Eco*RI-*Sal*I (B). The first lane shows the hybridization signal of one copy of *Eco*RI-digested pMCS DNA per genome. The two bands indicated by arrows are derived from the SV40 copy in the host genome, to which the SV40 *ori* part of the probe hybridizes. Numbers on the left side represent molecular weight markers.

resed vector DNA in amounts equivalent to one copy per genome on the same blot, it appears that on the average only a few pMCS vector copies are integrated into the XP12ROSV40 genome. We conclude, therefore, that UV damage in vector DNA does not drastically influence the amount of DNA integrated into the genome of the transformed cell.

DISCUSSION

This study demonstrates that UV-induced lesions in vector DNA stimulate its transformation efficiency. The UV dose range which gives an optimal stimulatory effect induces 20 to 150 dimers per pMCS dominant marker molecule, of which ca. 1/5 is estimated to be within the selectable gene itself. Evidently the deleterious consequences of this damage on the integrity and expression of the transfected gene are outweighed by enhancement of a step(s) in the transformation process. Moreover, the UV-induced lesions appear to be equally harmful in excision repair-deficient XP cells and wild-type cells, as judged from the fact that the transformation stimulation in XP-A cells is at least as high as in normal cells. Similar observations have recently been reported by Spivak et al. (41). These authors showed that UV irradiation of pSV2neo or pSV2gpt results in enhanced transformation frequency of human cells. The reported dose dependency and stimulation factor correspond well with our results.

The finding that similar stimulation is found with XP and wild-type cells contrasts with the survival of UV-damaged virus in XP cells, which is considerably lower than in normal cells (1, 2). The absence of an effect of the XP mutation on the transformation frequency of UV-irradiated vector DNA can be explained in different ways.

(i) The more deleterious effects of the UV lesions in XP cells are compensated by stronger stimulation of the step(s) in the transformation process which is influenced by the UV damage in the transfected DNA (e.g., because in XP cells the lesions exist for a longer period of time).

(ii) The XP-A cells repair the lesions in the exogenous DNA. Mortelmans et al. (34) and Kano and Fujiwara (25) have reported that extracts from various XP complementation groups (including XP-A and XP-F) are capable of excising pyrimidine dimers from purified DNA and from heterologous (but not homologous) chromatin. If XP cells can remove the dimers in the introduced DNA, this could explain the absence of a difference in UV stimulation of the transformation frequency. Further experiments are required to decide between these possibilities.

Which step in the transformation process is influenced by UV damage in DNA? The finding that initially unlinked DNA molecules eventually are integrated as a large concatenate into the host cell genome indicates that before or during incorporation frequent ligation and recombination events occur among transfected DNA molecules (32). In stable transformants the donor DNA is inserted at one (or a few) site(s) in the host chromosomal DNA (36), which suggests that the integration event might be one of the limiting steps in the transformation process. Since UV irradiation of cells, as well as transfection of cells with UV-irradiated DNA, enhance recombination (13, 21), it is possible that the UV stimulation of transformation is a consequence of the effect on recombination. UV light might stimulate recombination of exogenous DNA molecules with each other to form a concatenate and with the host DNA for stable integration. The following observations are consistent with this interpretation. (i) Stimulation of recombination is

also displayed by excision deficient XP cells (21). (ii) The enhancement of the recombination frequency by UV light is in the same order of magnitude as that of transformation and requires comparable doses of UV light (21). (iii) The experiments presented in Table 2 suggest that the presence of UV damage in the genome of the recipient cells also enhance the transformation frequency. This would indicate that UV damage in either one of the recombining DNAs results in stimulation of transformation. (The observation that XP cells exhibit higher stimulation than wild-type cells, after correction for cell survival, might be due to longer persistence of the UV lesions in the XP genome.)

Two types of recombination take place in the cell after transfection. The occurrence of homologous recombination between appropriately constructed exogenous DNA molecules is well documented (11, 14, 26, 35, 38, 39, 44). The recombination frequency is directly related to the size of the homologous segment between the transfected molecules (35, 39). Nonhomologous recombination between transfected DNAs has also been observed (3, 44). In this case small regions of partial homology between the donor molecules may play a role in the recombination process (3). In both types of recombination, replication of the transfected DNA is not required (44). As far as the interaction of exogenous DNA and host genome is concerned, it is known that integration can occur in any of a large number of sites in the host chromosomes (36). The lack of site specificity—even when ribosomal DNA segments, which have ca. 200 homologous gene copies in the mammalian genome (42), are transfected—strongly argues against homologous recombination as a predominant mode of integration. In our experiments the amount of homology between dominant marker and host genome has no effect on the level of UV stimulation of the transformation process. Therefore, if UV light exerts its effect on the transformation via enhancement of recombination between exogenous and host DNA, the latter must be of the nonhomologous type.

Southern blot analysis of DNA of XP12ROSV40 transformants obtained with dominant markers indicates that UV irradiation of the vector does not have a significant effect on the average number of marker molecules, nor on the amount of unirradiated cotransfected DNA (unpublished data) incorporated into the genome. The transformed XP12ROSV40 cells appear to have incorporated on the average one to two copies of the dominant marker. Compared with transfections with Ltk⁻ cells, which can integrate in the order of 7,000 kilobases (36), the amount of integrated DNA in XP12ROSV40 cells is extremely low. We are currently investigating whether the low amount of exogenous DNA stably inserted into the XP12ROSV40 genome is a unique feature of this cell line.

What is the inducing signal to the increase of transformation frequency? The experiments with pretreatment of UV-irradiated DNA with PRE, which acts specifically on dimers, indicate that pyrimidine dimers are responsible for this phenomenon, although the contribution of other, quantitatively less important UV lesions is not excluded. The finding that AAF mimics UV, whereas DNase and γ rays (at the doses tested) do not, suggests that stimulation of transformation is a property of lesions on which the excision repair pathway is operative. It is possible that the structure of the lesion itself (local deformation of the helix) produces stimulation of the transformation efficiency, e.g., by provoking recombination at those sites. Alternatively, UV-like lesions might induce some kind of SOS response in the transfected cell, one of the consequences of which is enhancement of

recombination and integration into the host genome. The fact that this occurs in XP-A and XP-F cells would indicate that these cells behave normally with respect to this part of the response and that a functional excision repair pathway is not required.

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