REPAIR AND REPLICATION OF ADENOVIRUS IN CHINESE HAMSTER OVARY CELL DNA REPAIR MUTANTS

EFFECTS OF THE DEN V GENE FROM THE BACTERIOPHAGE T4 AND THE HUMAN ERCC1 GENE ON THE REPAIR AND REPLICATION OF ADENOVIRUS IN MAMMALIAN CELLS

By

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ABSTRACT

The characterization of rodent cell mutants hypersensitive to UV light has led to the identification of at least 10 complementation groups all defective in some aspect of the first step in the excision repair of UV damaged DNA. The phenotypic properties of these mutants are thus of considerable importance to our understanding of DNA repair. In recent years five different excision repair cross complementing (ERCC) human genes have been isolated which correct the DNA repair deficiency in a number of Chinese Hamster Ovary (CHO) cell mutants and at least three of these genes also complement the repair deficiency in cells from patients suffering from xeroderma pigmentosum (XP), Cockayne syndrome (CS) and/or Trichothiodystrophy (TTD).

Adenovirus (Ad) infection of rodent cells is generally semi-permissive and does not give rise to viral progeny, such that Ad reactivation in CHO cells has not previously been reported. This study utilizes the ability of CHO cells and human cells to replicate viral DNA in order to examine the reactivation of Ad in several CHO as well as human cell DNA repair mutants. Unirradiated and UV-irradiated suspensions of Ad were assayed for their ability to synthesize viral DNA following the infection of several CHO and human cell DNA repair mutants. The cell types examined included CHO cell mutants from complementation groups 1, 2, 3, 4, 5, 6, 9, 10 as well as human XP and tumour cells. The survival of viral DNA synthesis for UV-irradiated Ad was significantly reduced in several of the CHO and human cell mutants compared to that in

normal cells. Cell mutants showing a reduced UV survival for this viral function included CHO cell mutants from complementation groups 1 to 6, XP cells and the 2 human tumour cell lines examined. This reduced host cell reactivation (HCR) for Ad indicates a reduced capacity for the repair of viral DNA in these cell types. DNA replication for unirradiated virus was also reduced for some of the mutants, especially the UV20 CHO cell mutant from complementation group 1, suggesting a deficiency for both DNA replication and repair in these cells.

This study also used the recombinant viruses Ad5(denV) and Ad5(ERCC1) as vectors to examine the effect of the bacteriophage T4 denV gene and the human ERCC1 gene on viral reactivation in the various cell mutants. UV survival of Ad5(denV) was increased compared to that of the control Ad5(LacZ) following infection of all the CHO and human cell types examined, indicating the denV gene product increases repair of Ad in both repair-proficient and repair-deficient cells. UV survival of Ad5(ERCC1) was increased compared to that of control Ad5(LacZ) following infection of the CHO mutant UV20 from complementation group 1, as well as all CHO cell types having normal HCR for Ad5. However, UV survival of Ad5(ERCC1) was not increased compared to Ad5(LacZ) following infection of CHO mutants from complementation groups 2 to 6 and 10. These results support a specific complementation of the UV20 repair defect by ERCC1 and suggest that the human ERCC1 gene is more efficient than its hamster counterpart in repair-proficient CHO cells or that the ERCC1 product is rate-limiting for the excision repair process in CHO cells. UV survival of Ad5(ERCC1) was also increased compared to Ad5(LacZ) in the normal human fibroblast cells and human tumour cells, but not in the XP (group D) cells.

The kinetics of viral DNA synthesis and viral protein synthesis for unirradiated Ad5(denV) and Ad5(ERCC1) was also investigated following the infection of human and rodent cells. The deficiency in viral DNA synthesis and viral protein synthesis found for Ad5(LacZ) following infection of rodent compared to human cells is partially complemented by either denV or ERCC1. The more marked deficiency in viral DNA synthesis of the UV20 CHO mutant was also complemented by either ERCC1 or denV, suggesting an ability of these genes to function in both repair and replication of viral DNA.

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LIST OF ABBREVIATIONS

CFA	- colony forming ability
D_0	- D 'zero' is the dose which results in, on average, one hit per target
D_{37}	- D 'thirty-seven' is the dose which reduces the surviving fraction to 0.37
denV	- Bacteriophage T4 endonuclease
ER	- enhanced reactivation
ERCC	- excision repair cross-complementing
ESS	- endonuclease sensitive sites
HCR	- host cell reactivation
PD	- pyrimidine dimer
REF	- replication enhancement factor
Vag	- viral structural antigens

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DEDICATIONS

This study is dedicated to Dad, Mom, Dean, Glen, Mary-Lynn, and Lance. You are the rock from which I draw my strength.

INTRODUCTION

The integrity of DNA, the basic blueprint of life, is constantly being challenged by damaging agents ranging from ionizing and non-ionizing radiation through an ever-growing number of potent chemicals (Friedberg 1985). Therefore, overcoming this genetic assault is of primary importance to living creatures so as to ensure individual as well as species survival. With this in mind, it is of little wonder that various strategies of dealing with DNA damage are ubiquitous among essentially all living organisms from the smallest prokaryotes through to the most sophisticated eukaryotes. The consequences of breakdown in these systems which repair or process damaged DNA are extremely deleterious and often fatal.

Indeed, abnormal processing of DNA damage has been implicated in a number of well documented human syndromes. Xeroderma pigmentosum (XP), ataxia telangiectasia (AT), Bloom's syndrome (BS), Cockayne syndrome (CS), and Fanconi's anemia (FA) are human genetic disorders which all demonstrate some aberration in the processing of damaged DNA (Cleaver 1968; Taylor *et al.* 1975; Hirschi *et al.* 1981; Marshall *et al.* 1980; Auerbach and Wolman 1978). There is speculation that this abnormal DNA damage processing may be linked to various cancers as individuals suffering from XP, AT, FA, and BS are more susceptible to certain cancers than healthy subjects (Setlow 1978; German *et al.* 1977). Our understanding of these disorders is ever-expanding and recent efforts to isolate and clone human DNA repair genes is testament to the importance which is attached to this field of study.

Whereas the study of human DNA repair pathways is still in its infancy, the knowledge accumulated in the area of bacterial DNA repair is providing valuable insights in the deciphering of the more sophisticated mammalian machinery. While the repair of DNA at the bacterial and mammalian levels appear to differ in their degrees of complexity, there are similarities which speak of evolutionary conservation and which are helping in the discovery of the specific functions of the mammalian DNA repair genes.

As the body of knowledge surrounding the processing of DNA damage increases, so does our chance of understanding the molecular basis of several human genetic disorders and the increased incidence of cancers associated with some of these disorders.

BACTERIAL DNA REPAIR

The repair of DNA damage can be accomplished through reversal of the damage or removal of the damage. Examples of these two modes of repair are photoreactivation and excision repair, respectively. DNA damage, if not repaired, may be bypassed by post-replication mechanisms and/or recombination.

Photoreactivation

In the late 1940s, Albert Kelner observed that subsequent to UV irradiation, the survival of samples of *Streptomyces griseus* spores exposed to light was 100 000 to 400 000 fold better than controls that were kept in the dark

(Kelner 1949). About the same time, Renato Dulbecco noticed a similar light dependency in samples of the T group of coliphages which were UV-irradiated and he coined the term 'photoreactivation' to describe it (Dulbecco 1949).

Cis-syn cyclobutyl pyrimidine dimers (PD) can be monomerized by an enzyme in a light-dependent process. The enzyme which catalyzes enzymatic photoreactivation (EPR) is known as DNA photolyase or photoreactivating enzyme (see Figure 1). DNA photolyase recognizes a PD and binds to that area of the DNA. The DNA-DNA photolyase complex then absorbs light with a wavelength greater than 300 nm which allows the monomerization of the dimer. Subsequent to the monomerization event, DNA photolyase is released. DNA photolyase has been detected in a broad spectrum of organisms including algae, bacteria, yeast, fungi, protozoa, molluscs, arthropods, teleosts, amphibians, reptiles, birds, marsupials, and placental mammals (Rupert 1975). However, catalytic activity of DNA photolyase has not been demonstrated in vivo for all of the cases enumerated.

EPR should also be distinguished from other light-dependent processes which do not rely on enzymes and which have wavelength requirements of 240 nm or less (Sutherland and Griffin 1980).

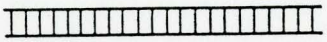
Excision Repair

Excision of damage to DNA can be accomplished via one of two modes (Friedberg 1985). Base excision repair removes inappropriate bases from the genetic code and results in their appropriate counterparts being re-introduced.

Figure 1: Photoreactivation of pyrimidine dimers

This series of panels describes the enzymatic photoreactivation of DNA containing pyrimidine dimers (PDs). DNA photolyase is able to bind specifically to the PD. Absorption of light with a wavelength greater than 300 nm results in the monomerization of the PD and the subsequent dissociation of DNA photolyase.

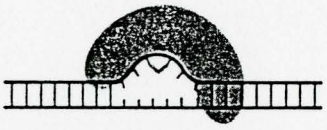
This figure was modified from Friedberg 1985.



Undamaged DNA

Pyrimidine dimer (PD)

introduced into DNA



Recognition of PD by

DNA photolyase

Absorption of light (> 300nm)

results in the monomerization of the PD

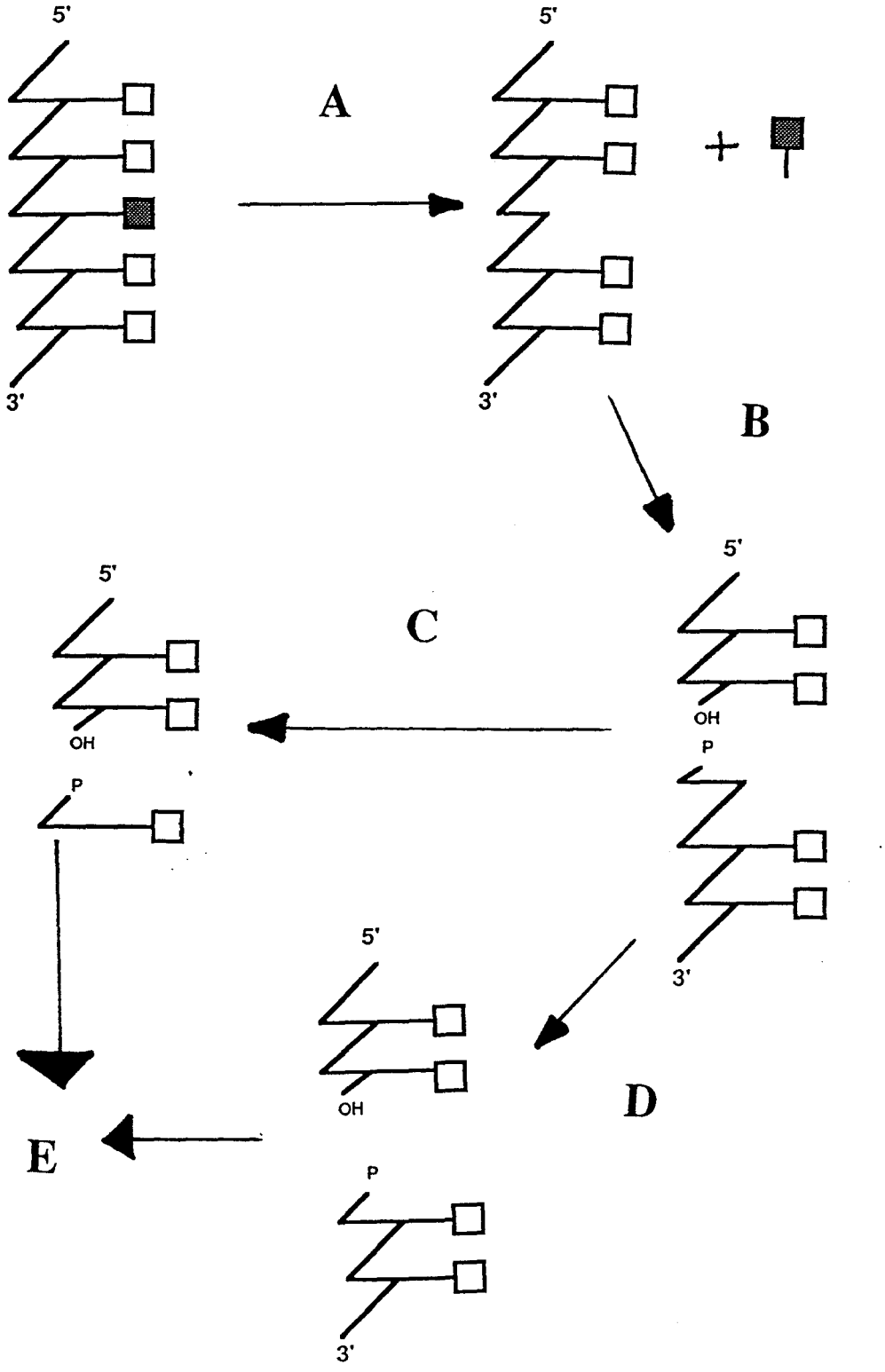
Native structure of DNA restored

DNA photolyase dissociates

Figure 2: Schematic of DNA base excision repair

An alkylated base (shaded square) is acted upon (A) by a DNA glycosylase resulting in an apurinic/apyrimidinic (AP) site being created. The next step (B) is performed by a 5' AP endonuclease which cleaves the deoxyribose-phosphate backbone at this AP site. A 5'-3' exonuclease (C) or a 3' AP endonuclease (D) is then able to act to remove the abasic region of the DNA backbone. DNA polymerase and ligase activities (E) are then involved in restoring the original DNA sequence.

This figure was adapted from Freidberg (1985).


5'
3'
A
5'
3'
+
B
5'
OH
P
3'
C
5'
OH
P
D
5'
OH
E
P
3'

Nucleotide excision repair removes bulky adducts from DNA and replaces the excised moiety with the correct sequence of nucleotides.

Base excision repair is mediated by DNA glycosylases and apurinic/apyrimidinic (AP) endonucleases. An example of this type of repair is presented in Figure 2. The first step is performed by a DNA glycosylase which catalyzes the hydrolysis of the N-glycosidic bonds which link the bases to the deoxyribose-phosphate backbone (Lindahl 1979). This reaction liberates the inappropriate base generating an AP site in the DNA. The next step may be accomplished by a 5' AP endonuclease which catalyzes the hydrolysis of phosphodiester bonds at the AP site resulting in 3'-OH and 5'-P termini. The subsequent action of either a 3' AP endonuclease or a 5'-3' exonuclease removes the baseless deoxyribose-phosphate backbone leaving a gap which is filled by the action of a DNA polymerase and a DNA ligase. The most characterized prokaryotic nucleotide excision repair pathway is the *Escherichia coli* Uvr nucleotide excision repair mechanism which repairs UV damage to DNA. The first step involves the dimerization of two UvrA protein molecules (Oh *et al.* 1989). One UvrB protein molecule then combines with $UvrA_2$ to form a damage recognition complex (Orren and Sancar 1989). Following damage recognition, a stable preincision complex is formed (Oh and Grossman 1989). The preincision complex is subsequently bound by UvrC protein which effects incisions at the 8th phosphodiester bond 5' to the lesion and at the 3rd or 4th phosphodiester bond on the 3' side of the lesion. UvrD (DNA helicase II) and DNA polymerase I are then involved in the release of the damage-containing oligonucleotide and the filling of the gap by DNA synthesis (Caron *et al.* 1985). Excision repair is completed by sealing of the nick by ligase. In vitro studies

have demonstrated stimulation of the UvrABC nucleotide excision repair by DNA photolyase (Sancar *et al.* 1984a).

Post Replication Recovery

Bulky base adducts are known to inhibit the replication of DNA in prokaryotes (Setlow *et al.* 1963; Hall and Mount 1981). This replication inhibition is temporary and one model put forth to explain the resumption of DNA replication on templates containing replication blocks is post replication recovery (Rupp and Howard-Flanders 1968; Howard-Flanders 1981). The model proposes that after DNA replication has been blocked by the lesion, DNA replication re-initiates downstream, leaving gaps in the daughter-strand opposite adducts. RecA protein is thought to bind to this single-stranded region causing it to align with a homologous section of the sister chromatid complex. Nicking of the undamaged parental strand, which is complementary with the partially synthesized daughter strand, permits a crossed-strand exchange. The gaps are then filled in and the strands are cut and rejoined.

SOS Repair In *E. coli*

In *E. coli*, the SOS repair system is induced by various DNA damaging agents and is under control of the RecA-LexA regulon (Little and Mount 1982). LexA binds to promoters of target genes and in doing so inhibits expression of these genes. It is thought that DNA damaging agents act to stimulate the activity of the RecA protein which then cleaves LexA. The cleavage of LexA obliterates

its repressor function allowing increased expression of the SOS genes. As the DNA damage is corrected, RecA becomes less active and LexA levels increase as it reassumes its transcription repressor role and shuts down the SOS response.

Included among the approximately 20 known genes under the influence of the SOS regulon are regulator genes (*lexA* and *recA*) (Little *et al.* 1981; Brent and Ptashne 1981; McPartland *et al.* 1980), excision repair genes (*uvrA*, *uvrB*, *uvrD*) (Kenyon and Walker 1981; Sancar *et al.* 1982), mutagenic bypass genes (*umuD*, *umuC*) (Bagg *et al.* 1981), cell division genes (*sulA*, *sulB*), and other damage inducible genes (*din*) (Kenyon and Walker 1980) whose functions are not yet known. In concert, the activated SOS system acts to increase excision repair, increase recombinational activity, increase mutagenic repair, and delay cell division; thereby increasing the odds for cell survival.

Mammalian DNA Repair

Photoreactivation

It is known that DNA photolyases are found in a wide variety of organisms. These enzymes, responsible for enzymatic photoreactivation, have been detected in human leukocytes and human fibroblasts (Sutherland 1974; Sutherland *et al.* 1974). Their proposed mode of damage reversal is similar to that of bacterial DNA photolyase as illustrated in Figure 1.

Excision Repair

Mammalian DNA excision repair is accomplished via base excision repair or nucleotide excision repair (Friedberg 1985). Base excision repair corrects damaged or inappropriate bases. It is mediated by DNA glycosylases and AP endonucleases and proceeds in a similar manner to that in bacterial cells (Figure 2). The first step involves a DNA glycosylase which generates an AP site by cleaving the glycosidic bond between the damaged base and the deoxyribose-phosphate backbone. Secondly, an AP endonuclease cuts one of the phosphodiester bonds of the AP site. This is followed by elimination of the AP section of the backbone by AP endonuclease or exonuclease activities. The final step is the filling in and sealing of the gap by polymerase and ligase functions.

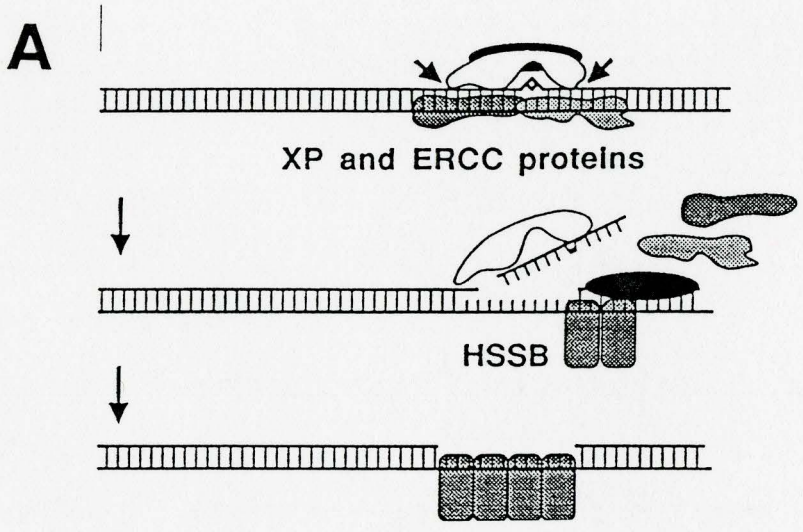
Mammalian nucleotide excision repair is a complex pathway as can be seen by the number of genes implicated solely in the incision step of this repair. At least 10 distinct human genes (Hoeijmakers 1991) and 10 rodent genes (Busch *et al.* 1989) are required for the incision step. This large number of genes required simply for incision is thought to reflect the higher order packaging of mammalian DNA. The accumulated evidence points to a two stage model of mammalian nucleotide excision repair as illustrated in Figure 3 (from Shivji *et al.* 1992). The first stage involves the cooperative involvement of the XP-A protein, additional XP proteins, and ERCC proteins to introduceincisions on either side of the bulky DNA adduct. Human single-stranded binding protein (HSSB) which is required for excision repair (Coverley *et al.* 1991) is thought to help displace the incised oligonucleotide and perhaps

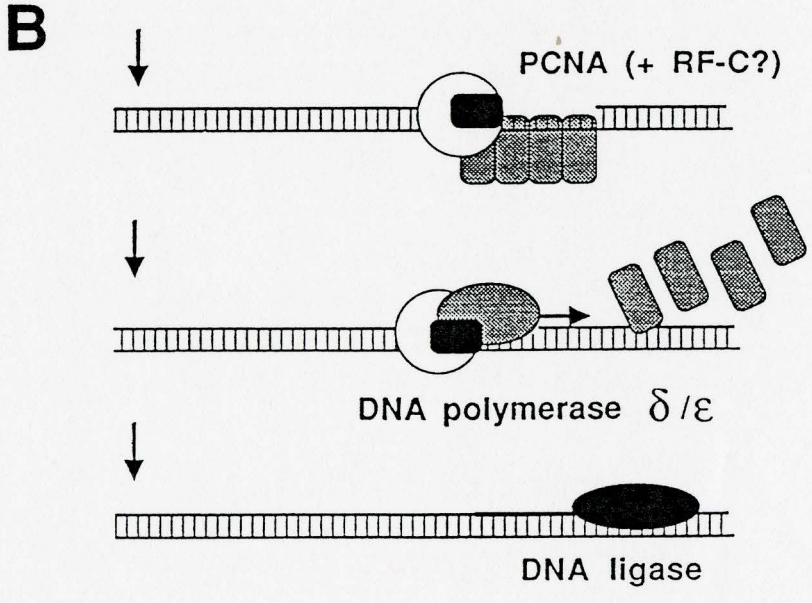
Figure 3: Schematic of mammalian nucleotide DNA excsion repair

The nucleotide DNA excision repair model is divided into two phases, the first is the incision step (A) and the second is all the post-incision events.

In A, the XP, ERCC proteins and perhaps others are involved in performing the incision step. The human single-stranded DNA binding protein (HSSB) is involved in removing the excised portion of DNA and in the protection of the incised DNA. The second (B) part of this pathway is dependent upon proliferation cell nuclear antigen (PCNA). The association of PCNA with DNA polymerases δ or ε is thought to reflect a role of these polymerases in repair synthesis. Lastly, ligase activity is involved in the sealing of the gap in the DNA.

This figure was adapted from Shivji *et al.* (1992).


A
XP and ERCC proteins
HSSB


B
PCNA (+ RF-C?)
DNA polymerase δ/ε
DNA ligase

protect the gapped region from cellular degradative enzymes. The second stage involves filling in of the gap created in stage one. This filling in is dependent on proliferating cell nuclear antigen (PCNA) (Shivji *et al.* 1992) which is known to be associated with DNA polymerase δ and ε, implying one or both of these polymerases in nucleotide excision repair synthesis. Finally, the gap is sealed by the action of DNA ligase.

Heterogeneous Repair of Mammalian DNA

An investigation of the removal of PDs from DNA following UV-irradiation of CHO cells showed that dimers were removed more efficiently from the actively transcribed DHFR gene than from the overall genome (Bohr *et al.* 1985). Experiments with human cells also revealed that the rate of repair of a number of actively transcribed genes in human cells is increased relative to that of the genome overall (Mellon *et al.* 1986). The rate of repair of the activelytranscribed mouse *c-abl* gene was also shown to be faster than that of the inactive mouse *c-mos* gene (Madhani *et al.* 1986). The preferential repair of actively transcribed genes was further dissected to show that removal of PDs from the transcribed DNA strand is much more efficient than that in the non-transcribed strand in both rodent and human cells (Mellon *et al.* 1987).

A model has been put forward to explain these results in terms of the nuclear organization of DNA in mammalian cells (Mullenders *et al.* 1991). The nucleoskeleton is proposed to be the active site of transcription and DNA replication. The observations of preferential repair of actively transcribed DNA are easily explained if DNA repair enzyme complexes are also associated with

the nucleoskeleton. The slower repair of bulk chromatin is a result of a reliance on diffusion of repair enzymes to sites of DNA damage. Therefore, the current model of eukaryotic DNA repair has three hierarchical levels:

1. The repair of active genes is relatively fast as a result of the localization of the machinery of DNA replication, transcription, and DNA repair to the nucleoskeleton.

2. The repair of the transcribed strand is more efficient than the untranscribed strand due to some unidentified mechanism.

3. The repair of transcriptionally inactive DNA is relatively slow (as in human cells) or greatly reduced (as in CHO cells) due to a reliance on simple diffusion of repair enzymes to chromatin not associated with the nucleoskeleton.

A comparison of DNA repair in CHO and human cells reveals that the repair of actively transcribed genes in the two species is similar. Removal of endonuclease sensitive sites (ESS) from the DNA of irradiated cells was used as an assay for DNA repair. Assessment 24 hours subsequent to a UV fluence of 5 J/m^2 to the cells indicated normal human cells removed 80% of ESS from the active DHFR gene while 70% were removed in CHO cells (Bohr *et al.* 1986). It is the repair of bulk chromatin that differs substantially between the two species. The level of repair in the overall genome, determined in this ESS assay, was found to be 85% and 15% for normal human cells and normal CHO cells, respectively.

DNA Repair Mutants

The isolation and characterization of cell mutants has proven to be a very successful strategy in the elucidation of various biochemical pathways. Researchers interested in the mechanisms of DNA repair have employed this approach with considerable success. Our present understanding of bacterial DNA repair as well as mammalian DNA repair has been achieved largely as a result of research with DNA repair mutants.

Bacterial DNA Repair Mutants

Many advances in E. coli genetic research have been brought about through investigations with various cell mutants. The UvrABC nucleotide excision repair in *E. coli* has been characterized in part due to work with *uvrA*, *uvrB*, *uvrC* (Howard-Flanders and Theriot 1962; Howard-Flanders *et al.* 1966) and *uvrD* mutants (Kuemmerle and Masker 1980). Additionally, *lexA* and *recA* genetic mutants have been helpful in achieving our present understanding of the bacterial SOS regulon (Mount *et al.* 1972; Gudas 1976).

Mammalian DNA Repair Mutants

Many of the investigations of mammalian DNA repair are being conducted with mutant cell lines defective in some aspect of this process. Human DNA repair cell mutants which are being used include some whose deficiencies have been laboratory-induced as well as some naturally-occurring

DNA repair mutants (Collins and Johnson 1987). Laboratory-induced rodent DNA repair-deficient mutants are also being used extensively in the research of mammalian DNA repair (Thompson *et al.* 1987).

Human DNA Repair Mutants

Human laboratory-induced DNA repair-deficient cell mutants have been isolated using various criteria. Two cell lines, S-1M and S-2M, were derived from HeLa S3 cells based on their incompetence for UV repair as determined by BrdUr suicide selection (Isomura *et al.* 1973). Both S-1M and S-2M are defective in dimer excision while only S-2M exhibits a cross-sensitivity to 4-NQO. Reduced host cell reactivation of herpes simplex virus was used successfully to obtain UVS-1 and UVS-2, two cell lines derived from human FL cells (Shiomi and Sato 1979).

Naturally-occurring human DNA repair-deficient mutant cell lines have been isolated from individuals afflicted with a number of genetic disorders including XP, BS, AT, CS, and FA.

XP is clinically characterized by severe photosensitivity of the skin and eyes, a high incidence of skin cancer, and neurological abnormalities (Robbins *et al.* 1974; Cleaver and Bootsma 1975). Systematic complementation analyses have to date established the existence of seven complementation groups (XP-A through XP-G). In addition to the seven complementation groups, there is also an XP variant. XP variant patients exhibit the classical symptoms of XP. However, cell lines derived from these individuals are similar to cell lines from normal individuals in levels of unscheduled DNA synthesis and the

extent of removal of endonuclease-sensitive sites (ESS) following UV-irradiation, two assays in which classical XP cells are deficient (Friedberg 1985). When comparing these two endpoints in the various XP complementation groups, there is a large degree of variation. The amount of repair synthesis, or unscheduled DNA synthesis, following UV-irradiation varies from a low of 0% to 10% of normal human fibroblast values in both XP-A and XP-F cells to a high of 40% to 60% in XP-E cells (Friedberg 1985). After a UV fluence of 3 J/m^2 to the cells, the loss of ESS by 32 hours after UV-irradiation ranges from 0% of normal human fibroblast values in XP-A cells to 60% in XP-F cells (Friedberg 1985; Zelle and Lohman 1979). In terms of these assays, XP-D cells are an intermediate XP group as they possess from 10% to 40% of normal repair synthesis capacity. The ability of XP-D cells to remove ESS is approximately 20% that of normal cells by 32 hours post-UV.

XP-C cells are particularly interesting as they are proficient in the repair of transcriptionally active DNA but have poor repair capacity for bulk DNA (Venema *et al.* 1990b; Kantor *et al.* 1990). CS cells exhibit the reverse characteristics in terms of repair of actively transcribed DNA. Recent work demonstrates that it is the repair of transcriptionally active DNA that is defective in CS cells (Venema *et al.* 1990a).

Rodent DNA Repair Mutants

DNA repair-mutants isolated from rodent cells, and in particular Chinese hamster ovary cells, have been extremely useful in DNA repair studies.

Attempts to isolate human DNA repair genes by DNA-mediated gene transfer of human DNA from repair competent cells into human DNA repair- deficient cells have proven unsuccessful. Poor integration of foreign DNA into most human cell lines, has been proposed as the reason for this lack of success (Hoeijmakers *et al.* 1987). As a consequence, studies utilizing CHO DNA repair-deficient cell lines have become more prevalent as these cells circumvent the problems with foreign DNA uptake. Furthermore, CHO cells have good growth and handling properties and DNA repair-deficient mutants are widely available (Collins and Johnson 1987). Complementation analysis of rodent DNA repair mutants has identified 10 DNA nucleotide excision repair complementation groups (Thompson *et al.* 1981; Thompson and Carrano 1983; Thompson *et al.* 1987; Zdzienicka *et al.* 1988; Thompson *et al.* 1988b; Stefanini *et al.* 1991). Members of all of these complementation groups are deficient in some aspect of the incision step of nucleotide excision repair, reminiscent of classical XP cell lines.

UV20 belongs to rodent excision repair complementation group 1. Isolated on the basis of its sensitivity to UV (Thompson *et al.* 1980), its D_0 for colony forming ability (CFA) following UV is reduced 4.25 times compared to the parental AA8. UV20 also exhibits an extreme sensitivity to mitomycin C (MMC) and a slight sensitivity to ethyl methanesulphonate (EMS). Its D_{37} for CFA after treatment with MMC and EMS is reduced 80-fold and 2-fold, respectively, when compared to AA8 (Thompson *et al.* 1980). Exposure of UV20 to ^{60}Co γ-rays under aerobic conditions did not decrease its CFA significantly compared to AA8, however, similar exposure under hypoxic conditions results in a 1.8-fold decrease in D_0 for CFA, compared to AA8 (Rubin and Whitmore 1985). A

combination of DNA synthesis inhibitors and an alkaline elution assay were used to determine the relative rate of DNA incision of UV20 following UV-irradiation. Subsequent to a fluence of 6 J/m^2, the incision rate of UV20 was determined to be 11% that of AA8 (Thompson *et al.* 1982b).

UV5 has been assigned to complementation group 2. Similar to UV20, its D_0 for CFA is 4.25-fold less than AA8 following UV-irradiation. The effect of MMC treatment on the D_{37} for CFA of UV5 is a 3.9-fold reduction compared to parental, much less dramatic than the eighty-fold decrease exhibited by UV20 (Thompson *et al.* 1980). EMS treatment of UV5 resulted in a 1.4-fold decrease in D_{37} for CFA, compared to control values. UV5 subjected to ^{60}Co γ-rays under aerobic conditions does not experience a decrease in CFA compared to AA8. The incision rate of DNA damage of UV5 cells was determined by alkaline elution following UV to be 5% of AA8 values (Thompson *et al.* 1982b). The authors did not consider this result to be significantly different than the 11% value obtained for UV20.

UV24 is a member of complementation group 3. UV-irradiation reduced its D_0 for CFA 5.5-fold compared to parental whereas its response to X-rays is similar to that of the parental (Busch *et al.* 1980). Treatment of UV24 cells with MMC reduced its D_{37} for CFA 3-4 times compared to AA8 (Hoy *et al.* 1985). UV24 was found to have 5% the incising capabilities of AA8 subsequent to UV exposure, similar to values for both UV20 and UV5 (Thompson *et al.* 1982b).

Complementation group 4 is represented by UV41. Its D_0 for CFA is 4-5 fold less than that of parental. This cell line is similar to UV20 in that it displays a marked sensitivity to DNA cross-linking agents such as MMC. D_{37} for CFA after MMC treatment is reduced 90-fold when compared to AA8 (Hoy *et al.*

1985). Alkaline elution measurements used to determine DNA incising rates revealed UV41 to have 5% the levels of parental (Thompson *et al.* 1982b).

UV135 represents complementation group 5 and exhibits a 4-5 fold reduction in D_0 for CFA following UV-irradiation, similar to UV20, UV5, UV24, and UV41. It is moderately sensitive to MMC damage and has a 3-4 fold decrease in D_{37} for CFA compared to parental after such treatment (Hoy *et al.* 1985). UV135 possesses 4% the incising ability of AA8 following UV, as determined by an alkaline elution assay (Thompson *et al.* 1982b). This incising ability is in the range of CHO mutants from groups 1 through 4.

UV61 represents complementation group 6 and its reduction in D_0 for CFA following UV is 2.8-fold (Thompson *et al.* 1987), intermediate between values for groups 1 to 5 and parental. UV61 is remarkable as its defect lies specifically in the incision of cyclobutane dimers and not in that of 6-4 photoproducts (Thompson *et al.* 1988a.)

There are no CHO representative cell lines in rodent DNA nucleotide excision repair complementation groups 7 or 8. Cell line V-B11, a derivative of Chinese hamster lung V79 cells, is a representative of complementation group seven (Zdzienicka *et al.* 1988). US31, a derivative of mouse lymphoma line L5178Y, has been assigned to rodent complementation group eight (Thompson *et al.* 1988b).

The CHO representative for complementation group 9 is named 7PV and is derived from the parental CHO-K1 $prol^-$. Its D_{37} for CFA following UV is 50% of parental values, much higher than the values reported for mutants from groups 1 to 6. Unscheduled DNA synthesis following UV-irradiation fluences of

10 J/m^2 is 30% that of the parental value whereas its D_0 value for UV is 66% of K1 values (Stefanini *et al.* 1989; Stefanini *et al.* 1991; Stefanini *et al.* 1982).

4PV is the CHO representative of complementation group 10. Its reduction in D_{37} for CFA is 40% of parental values, which is similar to 7PV values. It was derived from CHO-K1 prol- and following UV, it exhibits 59% of the UDS seen in parental cells. Its D_0 value is approximately 71% of parental values (Stefanini *et al.* 1991; Stefanini *et al.* 1989; Stefanini *et al.* 1982).

DNA Repair Genes

The isolation and cloning of DNA repair genes has been extremely helpful in the deciphering of these repair pathways. It has also helped in the understanding of the deficiencies that exist within DNA repair mechanisms in various genetic disorders which display aberrant DNA repair.

Bacterial DNA Repair Genes

The most characterized bacterial DNA repair pathway is that of nucleotide excision repair of UV-induced damage in *E. coli*. The three genes which encode the proteins required for the incision step of this type of DNA repair are *uvrA*, *uvrB*, and *uvrC*.

The *uvrA* gene has been mapped to 92 minutes on the E. coli map and encodes a protein of 940 amino acids with a predicted molecular weight of 103 874 (Husain *et al.* 1986). The protein contains putative nucleotide binding sites and zinc finger domains (Doolittle *et al.* 1986).

UvrB maps to position 17 on the E. coli map. The translated protein has a molecular weight of 76 118 and consists of 672 amino acids (Arikan *et al.* 1986; Backendorf *et al.* 1986).

The *uvrC* gene is located at map position 41.5. Efforts to determine the translational start site of uvrC were hampered due to an apparent blockage of the site (Sancar *et al.* 1984b). This study proposed that *uvrC* encodes a protein with a molecular weight of 66 038. However, further studies revealed the translational start site to be 66 base pairs upstream to that proposed by Sancar *et al.* (1984b) thus making the protein 22 amino acids larger than originally thought (Moolenaar *et al.* 1987).

Bacteriophage T4 Endonuclease (*denV*)

The original observation that bacteriophage T4 were twice as resistant to UV-irradiation compared to other phage was thought to reflect an absence of some factor in these phage (Luria 1947). It has since been demonstrated that the *denV* gene responsible for this increased UV-resistance encodes an endonuclease activity specific for thymine dimers (Friedberg and King 1971).

The *denV* gene codes for a protein of 138 amino acids with a predicted molecular weight of 16 078 (Valerie *et al.* 1984). The gene product has been shown to incise UV-damaged DNA, specifically at sites of pyrimidine dimers (Nakabeppu *et al.* 1982; Gordon and Haseltine 1980). The first step in the action of this enzyme is the cleavage of the 5' glycosyl bond of the dimer, followed by the scission of the intrapyrimidinic phosphodiester bond (Friedberg 1985; Lindahl 1982). It is probable that these reactions are performed

sequentially as they have been observed to be uncoupled (Weiss and Grossman 1987; Liuzzi *et al.* 1987). The enzyme locates PDs via one-dimensional diffusion along the DNA molecule (Dowd and Lloyd 1990) and there is some evidence to suggest that T4 endonuclease V must be in a dimeric state for this scanning to occur (Nickell and Lloyd 1991).

DNA repair replication in isolated nuclei from normal and repair-deficient XP-A human cells has been shown to be stimulated by denV protein following UV-irradiation (Smith and Hanawalt 1978).

The DNA repair-deficient CHO cell line, UV5, was transfected with the *denV* gene (Valerie *et al.* 1985). UV-resistance of the transfected cell line was found to be intermediate compared to UV5 cells and normal CHO AA8 cells. The extent of excision repair in the transfected cell line, as assayed by isopycnic sedimentation, was found to be similar to that of AA8 cells. A different study examined the removal of PDs from the actively transcribed DHFR gene and from downstream sequences in this *denV*-transfected cell line 8 hours following UV-irradiation (Bohr and Hanawalt 1987). A UV-sensitive CHO cell line removed less than 20% of the dimers from the DHFR gene and no PD removal was detected in the downstream sequences compared to approximately 70% PD removal from both locations in the *denV*-transfected cell line.

Cell survival of *denV*-transfected XP-A cells following UV-irradiation increased significantly compared to XP-A cells but did not reach that of normal cells (Valerie *et al.* 1987). Excision repair synthesis of endogenous DNA in the *denV*-transfected XP-A cells was similar to that in normal human cells whereas in XP-A cells, very little excision repair synthesis was detected.

Rates of PD removal in three XP-A cell lines stably transfected with *denV* were examined 24 hours after UV and were found to be, on average, 144% those found in a normal human cell line (Ley *et al.* 1989). The repair patch size of the denV-transfected cells was determined to be 13 to 18 nucleotides in size compared to 95 nucleotides in the repair-proficient cells.

Microinjection of 10^3 molecules per cell of synthetically produced denV protein into XP cells from complementation groups A, B, C, D, F, and G caused a stimulation of UDS (Yamaizumi *et al.* 1989). This measure of repair continued to increase with the dose of denV protein until repair levels characteristic of normal cells were reached at 10^4 molecules of protein. The addition of larger amounts of protein to XP or normal cells did not increase UDS under two hour labeling conditions. The addition of larger amounts of denV protein to normal human cells did, however, increase the rate of UDS if labeling times were shortened..

Repair-proficient murine fibroblasts were also stably transfected with the *denV* gene. These transfected cells were 2.5 to 4 times more efficient at removing PDs compared to control cells (Kusewitt *et al.* 1991). The repair patch size of the denV-initiated repair was found to be 6 nucleotides, on average, compared to 38 nucleotides for endogenous mechanisms. Lastly, the UV survival of the *denV*-transfected fibroblasts was not increased.

Another recent study examined the effect of *denV* transfection into repair-proficient human fibroblasts, repair-deficient XP fibroblasts, and wild-type CHO cells. Parallel experiments delivered denV protein to these cell types via liposomes (Kibitel *et al.* 1991). Both means of denV delivery increased the amount of PDs removed in all cell types. The presence of denV was found to

improve UV survival of XP-A fibroblasts but did not help that of repair-proficient human fibroblasts or wild-type CHO cells.

A recombinant adenovirus (Ad) containing the *denV* gene in the deleted E3 region of the virus has also been constructed. The UV-survival of viral antigen formation (Vag) following infection of XP-A, XP-C, XP-E, and normal fibroblasts was determined (Colicos *et al.* 1991). UV-survival of Vag was increased in XP cells from all three complementation groups following infection with the *denV*-containing virus compared to control virus. There was not any increased UV-survival of Vag for normal fibroblasts infected with the Ad-*denV* vector.

The *denV* gene was also introduced into a herpes simplex virus type 1 expression vector (Tang 1991). The measure of repair in this study was UV survival of plaque formation. It was found that denV increased survival of plaque formation in XP cells from groups A, C, and D but was not detectable in XP-E or XP-F.

Mammalian DNA Repair Genes

Within the last few years, a number of mammalian DNA excision repair genes have been cloned. These include the XP-A complementing gene (*XPAC*), one X-ray repair cross complementing gene (*XRCC1*) and 5 excision repair cross complementing (ERCC) genes. The human XRCC and ERCC genes were so named because they were isolated on the basis of their ability to correct DNA repair defects in rodent cells.

The human *XPAC* gene, assigned to human chromosome 9q34.1, has been cloned (Tanaka *et al.* 1990). Two human XPAC mRNA, 1.3-1.4 kb and 1.0-1.1 kb, have been detected in normal human cells and are thought to be the result of alternative polyadenylations. A cDNA encoding a protein of 273 amino acids with a predicted molecular weight of 31 000 has been shown to correct the UV-sensitivity of XP-A cells. XPAC protein has been shown to contain a zinc-finger domain suggesting DNA binding activity.

The *ERCC2* gene which is approximately 19 kb has been assigned to human chromosome 19q13.2-13.3. It codes for a protein of 760 amino acids which corrects the DNA repair deficiency of UV5 and other CHO mutants of complementation group 2 (Weber *et al.* 1988). A cloned *ERCC2* cDNA of about 2.6 kb has also been shown to complement the UV-sensitivity of CHO UV5 cells. The ERCC2 repair protein exhibits a high degree of homology with yeast RAD3 protein (Weber *et al.* 1990) suggesting an ATP dependent helicase function. It is also postulated that *ERCC2* may play a role in DNA replication and is essential for cell viability. Two genomic *ERCC2*-transfectants of UV5 cells, 5T4-12 and 5C24-2, exhibited PD removal equivalent to that of parental AA8 cells (Regan *et al.* 1990). An ERCC2-bearing cosmid has been shown to specifically correct the UV-sensitivity of XP group D cells (197Flejter et al. 1992). UV survival studies with various XP groups have indicated that full length *ERCC2* cDNA is able to specifically correct the repair deficiency of XP-D cells (Weber *et al.* 1991).

The gene that corrects the repair deficiency of CHO complementation group 3 cells, such as UV24, is *ERCC3* (Weeda *et al.* 1990). It is located on human chromosome 2q21. Both genomic and cDNA clones of ERCC3 have

been cloned (Weeda *et al.* 1990). The encoded protein is 782 amino acids in length with possible nucleotide, chromatin, and helix-turn-helix binding domains. It also has homology to a number of helicases suggesting such an activity. *ERCC3*, as *ERCC2*, corrects the defect from one of the XP complementation groups. *ERCC3* is specific in its correction for XP-B. In addition, this XP-B patient also exhibits the symptoms of Cockayne's syndrome (CS). Consequently, *ERCC3* has been shown to correct the repair deficiency of CS-C indicating a role for *ERCC3* in the repair of transcriptionally active DNA (Venema *et al.* 1990a).

The gene which corrects the repair deficiency of rodent cells from complementation group 4, such as UV41, has been localized to human chromosome 16. Unfortunately, efforts to clone the gene have been unsuccessful thus far (Dulhanty *et al.* 1988).

The genomic version of *ERCC5*, which corrects the repair deficiency of UV135, has been cloned (MacInnes and Mudgett 1990). To date, a cDNA has not been isolated. Studies of the structure of *ERCC5*, which is located on human chromosome 13, have failed to suggest a specific function of the protein.

The specific defect in PD repair of the moderately UV-sensitive CHO cell line, UV61, is corrected by *ERCC6* which has been assigned to human chromosome 10q11-21 (Troelstra *et al.* 1990; Troelstra *et al.* 1992). The ERCC6 gene covers approximately 100 kb. A cDNA clone which approximates 75% of the smallest ERCC6 mRNA spans 80 kb of the cloned genomic locus. *ERCC6* has also been shown to correct the defect in the repair of actively transcribed DNA in CS-B cells. The protein encoded by this gene is predicted to consist of 1493 amino acids.

The CHO mutant EM9 demonstrates an increased sensitivity to ethyl methanesulfonate and to ionizing radiation (Thompson *et al.* 1982a). The human gene which corrects these aberrations, *XRCC1*, has been cloned and it encodes a protein of 633 amino acids with a molecular weight of 69 500. A partial cDNA clone lacking 26 nucleotides corrected EM9 cells to 80% of wild-type levels.

Excision Repair Cross Complementing Gene 1 (*ERCC1*)

The human *ERCC1* gene was identified by virtue of its ability to correct the UV-sensitivity of an excision repair deficient CHO mutant. The gene was isolated following transfection of repair-competent genomic HeLa DNA into the CHO mutant cell line, 43-3B (Westerveld *et al.* 1984). Following UV irradiation or MMC treatment, cell survival of the cosmid transfectants in the study approached, but did not attain, survival levels of wild-type CHO cells. UDS, as measured by ^{3}H-thymidine incorporation after UV-irradiation, increased in the transfectants to levels which surpassed that in the wild-type CHO cells.

The size of the *ERCC1* gene is approximately 15 kb. *ERCC1* has been assigned to human chromosome 19q13.2-13.3 and as such is one of three human DNA repair genes, the other two being ERCC2 and XRCC1, to be found on this chromosome (Mohrenweiser *et al.* 1989). Transcription of *ERCC1* results in multiple transcripts ranging from 1.1 kb to 3.6 kb. While the 1.1 kb transcript is subject to alternative splicing of an internal 72 bp coding region, only the 1.1 kb cDNA was able to correct the UV- and MMC-sensitivity of CHO complementation group 1 43-3B cells (van Duin *et al.* 1986). This correction of

the defect in rodent complementation group 1 cells has been shown to be specific for that group as transfection of ERCC1 into members of complementation groups 2 to 6 did not increase UV survival of those mutants (van Duin *et al.* 1988a).

The first 214 N-terminal amino acids of ERCC1 bear significant homologies to the 210 amino acid RAD10 of yeast, suggesting a DNA binding function for ERCC1. In addition, the C-terminus of ERCC1 bears some homology with two of the excision repair genes from E. coli, uvrA and uvrC (van Duin *et al.* 1986). Interestingly, amino acids 12-23 of ERCC1 protein bear significant homology with the nuclear location signal of SV40 large T antigen. Mutation studies utilizing a series of *ERCC1* cDNA constructs with various deletions have demonstrated that the highly conserved C-terminus is essential for correction of the UV-sensitivity of 43-3B cells whereas the N-terminal region is dispensable for this function (van Duin *et al.* 1988b).

Following transfection of the*ERCC1* gene into the repair deficient 43-3B CHO cell line, it has been shown that preferential repair of the actively transcribed dihydrofolate reductase gene and its flanking non-transcribed sequences is similar to DNA repair levels in wild-type CHO cells (Bohr *et al.* 1988).

Increased levels of the *ERCC1* 1.1 kb transcript have been reported in lymphocytes from patients with chronic lymphocytic leukemia resistant to nitrogen mustards (Geleziunas *et al.* 1991). This suggests that *ERCC1* is involved in repairing the alkylating damage induced by nitrogen mustards. It also implies that increased levels of *ERCC1* may in part be responsible for the

induced drug resistance of certain cancer cells following at least one type of drug therapy.

Adenovirus Infection

Adenoviruses belong to the family Adenoviridae which is divided into the two genera, Mastadenovirus and Aviadenovirus (Norrby *et al.* 1976). The Mastadenovirus genus includes opossum, canine, ovine, porcine, equine, bovine, simian, and human viruses.

An adenovirus is a nonenveloped, regular icosahedron (Horne *et al.* 1959) with a diameter of 65-80 nm. A fiber, the length of which depends on the viral serotype, projects from each of the 12 vertices.

Infection of Permissive Cells

The replicative cycle of adenoviruses is divided into early and late phases. The early phase consists mainly of the expression of early Ad genes from input adenovirus templates before the onset of viral DNA replication. There are four principle regions of the Ad genome which contribute to early (E) functions. The E1 region, which is subdivided into E1A and E1B, codes for more than 10 proteins which have a number of roles including transcriptional activation of other viral proteins. The E2 region encodes the three viral genes necessary for Ad DNA replication. E2A codes for a single-stranded DNA binding protein (DBP) with an apparent molecular weight of 72 kd which is made in large amounts in Ad-infected human cells (Linne *et al.* 1977). E2B

codes for the other two viral proteins involved in viral DNA replication. These are the 80 kd precursor terminal protein (pTP), which is cleaved to a 55 kd terminal protein (TP) during viral replication, and the 140 kd Ad DNA polymerase (Friefeld *et al.* 1983; Lichy *et al.* 1982). This polymerase shares five regions of partial homology with a group of polymerases including DNA polymerase a, HSV DNA pol, and the prokaryotic ϕ 29 DNA pol. The E3 region is nonessential for viral propagation in tissue culture and functions in the modulation of host response to Ad infection. The largest species encoded by E3 is the 19 kd protein which has 2-4 N-linked glycosylation sites and binds to the MHC polypeptide heavy chain in the endoplasmic reticulum (ER) and prevents the transport of the MHC polypeptides to the cell surface (Bachenheimer and Darnell 1975; Paabo *et al.* 1986; Persson *et al.* 1979). Polypeptides with apparent molecular weights of 14.7 and 10.4 kd are also encoded by E3. The functions of these two proteins are the inhibition of the lysis of Ad infected cells by tumour necrosis factor (TNF) and the binding of epidermal growth factor receptor, respectively (Gooding *et al.* 1988; Carlin *et al.* 1989). The E4 region codes for a number of proteins including an 11 kd protein which binds to the nuclear matrix (Sarnow *et al.* 1982) and a 34 kd protein that forms a complex with the E1B-encoded 55 kd protein and is located in the nucleus (Cutt *et al.* 1987).

The onset of viral DNA replication, which signals the beginning of the late phase of adenoviral infection, begins 6-9 hours post-infection in Hela cells (Horwitz *et al.* 1973; Pearson and Hanawalt 1971). Ad DNA is a linear duplex molecule with a unique region of inverted terminal redundancy of approximately 100 base pairs (Arrand and Roberts 1973). The inverted terminal redundancy

allows molecules to circularize by means of a panhandle structure which is identical to the end of the original duplex molecule (Garon *et al.* 1972; Wolfson and Dressler 1972). Each DNA strand elongates by a continuous mechanism in the 5'-3' direction and does not require 'Okazaki' intermediates (Horwitz 1971; Lechner and Kelly 1977). Rekosh *et al.* (1977) were the first to propose a protein-priming model of viral DNA replication in which replication begins at the ends of the viral strands. This hypothesis has subsequently been validated (Lichy *et al.* 1981).

The replication of adenoviral DNA requires three viral gene products and four host factors (Horwitz 1990). The E2 region encodes the necessary viral proteins. These are the 80 kd pTP, the 72K DBP, and the 140 kd DNA pol. The host factors required are nuclear factor I (NFI), NFII, NFIII, and ORP A.

The late phase of Ad infection begins with the onset of viral DNA replication, however, the controls which govern this switch are not well understood. The majority of virus-coded proteins that are synthesized late are viral structural proteins or their precursors. Interestingly, some early proteins such as the 72K DBP continue to be produced even after the infection has advanced into the late stages. Conversely, there are examples of late proteins which are also synthesized in the early stages, albeit in smaller amounts.

Infection of Semi-permissive and Non-permissive Cells

A normal lytic cycle of adenovirus includes expression of early viral genes, viral DNA replication, and expression of late viral genes which all culminate in the production of infectious virus particles. When cells are non-

permissive for Ad infection, infectious virus is not produced. Infection of non-permissive cells is characterized by variable early gene expression and the absence of viral DNA replication and late viral gene expression with no viral progeny evident. Infection of semi-permissive cells results in some degree of expression of viral genes with at least a 3 log reduction in viral progeny production.

Rodent cells are semi-permissive for infection with class C adenoviruses which include Ad2 and Ad5. Longiaru and Horwitz (1981) report that viral DNA synthesis is delayed in CHO cells following infection of cells with 4000 virions of Ad 2 per cell. They note, however, that viral DNA accumulates to normal levels when assessed at 40 hours, 60 hours, and 80 hours post-infection. More recently, the peak in viral DNA synthesis of infected CHO cells has been narrowed down to between 24 and 36 hours post-infection (Radna *et al.* 1987) in contrast to a permissive HeLa infection where the peak occurs 10-15 hours post-infection. Analysis of CHO cell lysates by PAGE was unable to discern any late viral structural proteins suggesting that the block to the production of infectious Ad particles in CHO cells lies between viral DNA replication and synthesis of late proteins (Longiaru and Horwitz 1981). Eggerding and Pierce (1986) report that following Ad2 infection of CHO-K1 cells, the synthesis of the E2A encoded 72K DBP is delayed but eventually reaches levels similar to a permissive infection. The amount of viral DNA synthesized in CHO cells by 48 hours is similar to that in HeLa cells when visualized by EtBr staining whereas the amounts of late structural proteins are vastly reduced.

Therefore, current data suggests that infection of semi-permissive CHO cells with class C adenoviruses is characterized by delayed synthesis of at least

one early gene (E2A 72K), delayed synthesis of viral DNA, and little or no synthesis of several late viral structural proteins. The basis of the restricted viral functions has been proposed to be a lack of expression of some cellular factor in CHO cells which is normally present in permissively infected human cells. An alternative explanation is that these cellular factors, although present in semi-permissive cells, may not be interchangeable in terms of the functions performed by their permissive counterparts. Studies with Ad infected human/CHO hybrids containing various complements of human chromosomes have suggested that human chromosomes 1 and 3 may encode factors necessary for overcoming the block in CHO cells (Radna *et al.* 1987).

Adenovirus As A Probe For DNA Repair In Mammalian Cells

Infection of bacterial cells with phage has long been used as a means of understanding bacterial DNA repair processes (Weigle 1953; Rupert and Harm 1966). In an analogous manner, a number of nuclear replicating DNA viruses, including adenovirus, have been very useful in the study of mammalian DNA repair mechanisms. Host cell reactivation, cellular capacity, enhanced reactivation, and mutagenic DNA repair are assays in which virus has been used in the investigation of mammalian DNA repair mechanisms.

Host Cell Reactivation (HCR)

HCR measures the ability of the host-cell to reactivate DNA-damaged virus. The extent of reactivation is a reflection of constitutive levels of repair in

that cell line. A variety of different endpoints may be used to assess HCR in cell culture experiments.

Plaque forming ability (PFA) has been used extensively as an endpoint of viral function. Infection of normal human fibroblasts, XP fibroblasts and XP heterozygote fibroblasts reveals that fibroblasts from XP complementation groups A and D were least capable of HCR of PFA (Day 1974). XP-B and XP-C were somewhat more capable of HCR in this assay. Normal fibroblasts and XP heterozygotes had the highest levels of HCR and were found to be indistinguishable in that respect. A reduced HCR of PFA following infection with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-damaged adenovirus type 5 was reported in several human brain tumour cell lines.

The formation of viral structural antigens (Vag) as determined in an immunofluorescence assay is a very sensitive means of investigating DNA repair capabilities of various cell lines. An examination of the HCR of Vag in XP heterozygotes subsequent to infection with UV-irradiated Ad2 demonstrated the heterozygous cell strains to be intermediate in HCR compared to normal strains and homozygous XP strains (Rainbow 1980). The HCR of Cockayne syndrome cell lines infected with UV-irradiated or γ-irradiated Ad2 shows a reduced HCR in both cases (Rainbow and Howes 1982). The Vag HCR assay was also used to compare the relative sensitivities of CS fibroblasts and XP fibroblasts to sunlamp, UV and γ-irradiation (Rainbow 1989a). The HCR values of three CS strains were lowest for UV, intermediate for g-irradiation, and close to that of normal fibroblasts for sunlamp-irradiation. The XP strains tested, representing complementation groups A, C, and D, demonstrated greatest sensitivity to UV, but intermediate sensitivity to sunlamp-irradiation, and highest HCR values for

γ-irradiation. Rainbow (1989c) compared the HCR of Vag of five human tumour cell lines, one SV40-transformed human cell line, and one Ad-transformed human cell line. The %HCR, compared to normal fibroblasts, of the tumor cell lines varied from a low of 26% for the colon carcinoma-derived HT29 cell line to a high of 53% for the melanoma M6 cell line. The SV40-transformed GM637 cell line was also found to have a reduced HCR value of 53%. The Ad5-transformed 293 cell line, however, did not exhibit any defect in DNA repair in this assay. An examination of a number of cell lines derived from individuals with BS, AT, and Huntington's disease (HD) did not reveal a decrease in HCR of Vag following exposure to sunlamp irradiation (Rainbow 1991). However, a small reduction in HCR of Vag was noted in HD cells after Ad exposure to far UV (Rainbow and Howes 1980). HCR values for Vag in XP-A fibroblasts, infected with γ-irradiated Ad2, were shown to be significantly lower when virus was irradiated at -75°C compared to 0°C (Bennett and Rainbow 1989).

Cellular Capacity

Cellular capacity assays are performed by examining the ability of cells treated with DNA-damaging agents to support subsequent viral infection. It is noteworthy that the action spectrums for bacterial and mammalian cell death following exposure to UV are superimposable upon the absorption spectrum of DNA for UV (Gates 1930; Todd *et al.* 1968; Coohill 1986). These observations support the view that the lethal effects of UV to bacterial and mammalian cells result from damage to the DNA. Thus, a capacity assay reflects the ability of a

cell to repair regions of its DNA which encode products necessary for viral infection.

PFA following infection of UV-irradiated XP fibroblasts with HSV has been shown to be reduced relative to that in normal fibroblasts (Lytle *et al.* 1976; Coohill 1981). A more recent study by Ryan and Rainbow (1986) demonstrated a reduction in capacity for HSV PFA of four CS cell strains to levels 26% to 30% that of normal human cell strains. HSV PFA capacity in the same study was determined to be 3%, 11%, 14%, and 63% of normal fibroblast levels for cell strains from XP-A, XP-C, XP-C, and XP-variant, respectively. A reduced capacity to support HSV PFA following UV-irradiation was found in human cell lines deficient in thymidine kinase (Rainbow 1989b). The HSV PFA capacity assay has also been used to demonstrate differential responses to 254 nm UV-irradiation and simulated solar-irradiation (Gill and Coohill 1987). The effect of exposure to 254 nm UV-irradiation on HSV PFA capacity in various cells was assayed. Reduction to 10% following UV-irradiation in African green monkey kidney cells, Bloom's syndrome fibroblasts, XP-C, XP-A, and XP-variant required exposure levels that were 67%, 59%, 71%, 17%, and 100% of exposure to normal cells, respectively. Exposure required to achieve reduction of capacity to 10% following solar simulated-irradiation was 176%, 83%, 107%, 83%, and 79% in African green monkey kidney cells, Bloom's syndrome fibroblasts, XP-C, XP-A, and XP-variant, respectively, compared to normal cells.

Enhanced Reactivation (ER)

The first indication of inducible repair mechanisms was noted in bacteria. Weigle (1953) observed that survival of irradiated phages was significantly increased when inoculated onto irradiated bacteria. This effect has become known as Weigle reactivation.

Mammalian cells treated with DNA damaging agents have also been shown to exhibit similar inducible phenomena which are known as enhanced reactivation. A molecular link has not yet been demonstrated between the bacterial and mammalian phenomena and as a result, their designations remain distinct.

HSV pretreated with UV have been shown to have increased plaque formation ability in CV-1 monkey cells (Bockstahler and Lytle 1970). Human HeLa cells have also demonstrated a UVER of UV-irradiated HSV in terms of plaque formation (Lytle *et al.* 1974) Rat mammary tumour cells exhibited UVER for viral UV doses greater than 435 erg/mm^2 and γ-ray ER following infection with UV-irradiated herpes virus (Hellman et al 1974). Interestingly, normal rat cells did not exhibit ER following either treatment. UVER of UV-irradiated Kilham rat virus was found to exist in rat nephroma cells (Lytle 1978). UVER for PFA of HSV was found in three CS cell strains and XP cell strains from groups A, C, D, and a variant (Ryan and Rainbow 1986). Bennett and Rainbow (1988a) report UVER of UV-irradiated Ad in normal human fibroblasts. The increased reactivation was approximately 3-fold. The phenomenon of γ-ray ER was found to be absent in AT cells compared to normal fibroblasts (Jeeves and Rainbow 1986). This absence of ER in AT fibroblasts has been proposed to be related to

the radioresistance of unirradiated virus expression in AT cells. A more recent investigation of AT cells demonstrated that UVER factors for UV-irradiated Ad in AT cells were below that of normal cells when infection occurred immediately after UV-irradiation of cells (Bennett and Rainbow 1988). The UVER factors for AT cells returned to near normal levels if the infection was delayed for 24 hours after irradiation of the cells.

Mutagenic DNA Repair

The survival of virus in cells which have been pre-treated with DNA damaging agents is known to be enhanced. If the endpoint chosen for this type of experiment is mutation of viral genes, one is able to assess the mutagenic, or error-prone, potential of these induced repair pathways.

Activation of the SOS regulon in *E. coli* results in the induction of a number of genes belonging to various repair pathways (Friedberg 1985; Little and Mount 1982). Some of these induced DNA repair pathways are known to be error-prone. The first report of a similar inducible error-prone phenomenon in mammalian cells was seen in UV-irradiated Vero cells which were infected with UV-irradiated HSV (DasGupta and Summers 1978). The frequency of mutations in the viral thymidine kinase gene was increased in UV-irradiated cells compared to unirradiated cells for both UV-irradiated and unirradiated HSV. An investigation of UV-enhanced mutagenesis (UVEM) was done in normal human fibroblasts infected with UV-irradiated temperature-sensitive Ad mutants (Bennett and Rainbow 1988a). The increased mutagenesis found in UV-irradiated compared to non-irradiated fibroblasts supports the notion of UV-

inducible error-prone mechanisms in normal human fibroblasts. A similar study with AT fibroblasts noted that UV-irradiation of AT cells prior to infection with UV-irradiated Ad resulted in a decrease in mutagenesis, suggesting that AT cells lack the inducible error-prone repair found in normal fibroblasts (Bennett and Rainbow 1988b).

Viral Vectors

The use of recombinant viruses for the delivery and the expression of foreign genes in various cell types is an area that has generated considerable interest in the last few years (Berkner 1988). This approach is providing an alternative to techniques such as $CaPO_4$ transfection and electroporation as a means of expressing foreign genes in cell culture. Recombinant viruses which express foreign antigens are also being investigated as a means of immunization.

Several different viruses, including vaccinia virus (Blancou *et al.* 1986; Rupprecht *et al.* 1986) and adenovirus (Ad) (Graham and Prevec 1991), are being explored as vectors for the delivery and expression of foreign genes in both tissue culture and immunization experiments.

Adenoviral Vectors

Since their isolation almost 40 years ago (Rowe *et al.* 1953), the study of adenoviruses has provided a wealth of knowledge concerning various aspects of cellular metabolism such as mRNA splicing (Berget *et al.* 1977) and initiation

of eukaryotic DNA replication (Challberg and Kelly 1979). As a result, the body of information concerning adenovirus infection has expanded considerably. This knowledge is now being exploited to deliver foreign genes, via Ad recombinant vectors, in tissue culture experiments as well as in *in vivo* immunization studies.

The Ad vector system has a number of inherent advantages including the capacity to insert up to 4 kb of heterologous DNA into helper independent viruses or 7 kb into helper dependent viruses (Graham and Prevec 1991). High levels of protein expression of foreign genes and a wide host range are also among the advantages of Ad vector systems.

A large number of adenovirus recombinants expressing heterologous genes have been reported in the literature. These include recombinants which carry genes encoding vesicular stomatitis virus (VSV) glycoprotein (Schneider *et al.* 1989), herpes simplex virus (HSV) glycoprotein (Johnson *et al.* 1988), hepatitis B virus surface antigen (Morin *et al.* 1987), rabies glycoprotein (Prevec *et al.* 1990), HIV 1 glycoprotein (Dewar *et al.* 1989), polyoma (Py) large T antigen (Massie *et al.* 1986), Py middle T antigen (Davidson and Hassell 1987), and the dihydrofolate reductase mouse gene (Ghosh-Choudhury and Graham 1987).

More recently, this type of recombinant viral vector strategy has been used as a means of studying DNA repair genes in repair-competent and repair-deficient cell lines (Colicos *et al.* 1991; Tang 1991). An Ad 5 vector containing the denV gene in the deleted E3 region of the virus has been constructed. The denV gene has been shown to help the HCR of Vag following UV in XP group A, XP group C, and XP group E fibroblasts but not that of fibroblasts from normal

individuals or those from individuals with Cockayne's syndrome (Colicos 1988). More recently, a herpes simplex virus type I vector carrying the denV gene in the non-essential glycoprotein I gene has been constructed (Tang 1991). Results from that study indicate that plaque formation ability of HSV(denV) following UV survives better than control virus in trichothiodystrophy cells, and in cells from XP complementation groups A, C, and D. Cells from XP complementation groups E and F as well as mouse L cells did not exhibit any increased UV survival for HSV(denV).

This study is the first one to report on the HCR of Ad in rodent, and specifically, Chinese hamster ovary cells. This knowledge represents an important addition to the field of DNA repair as DNA-mediated gene transfer into DNA repair-deficient CHO cells is the most successful route being used to isolate and clone human DNA repair genes at the present time. The Ad HCR information gathered from the study of these DNA repair-deficient mutants aids in the understanding of the human DNA repair genes which are able to correct the mutations in these cells.

The endpoint used in this assay was viral DNA synthesis. This endpoint has the advantage of being closely tied to DNA repair and as such is a truer reflection of DNA repair than some of the traditional endpoints such as plaque formation, which is quite far removed from the repair event. In addition, the use of viral DNA synthesis as an endpoint allows the investigation of a number of cell types, such as CHO, which are semi-permissive for Ad infection and would not result in plaque formation. The Ad HCR information gathered from the study

of these DNA repair-deficient mutants aids in the understanding of the human DNA repair genes which are able to correct them.

In addition to the use of adenovirus as a probe of cellular repair capabilities, this study has used the vectorology of Ad in the study of two DNA repair genes. Ad vectors containing the prokaryotic *denV* gene from bacteriophage T4 and the human DNA repair gene *ERCC1* were used in this study to examine the complementation of repair deficiencies in a range of CHO repair-deficient mutants as well as repair-proficient cell lines. The UV survival of Ad5(denV) was shown to be increased in all the repair-deficient CHO cells as well as the repair-proficient parental cells. The Ad5(ERCC1) vector specifically corrected the repair deficiency of UV20, a member of the rodent excision repair-deficient complementation group 1, but not that in any of the other UV-sensitive CHO cell lines which exhibited decreased UV survival. The UV survival of Ad5(ERCC1) was also increased, relative to control virus, in parental cells and other cell lines which exhibited parental UV repair capabilities.

MATERIALS AND METHODS

A. CELL LINES AND MANIPULATIONS

A.1 Parental Rodent Cell Lines

AA8 is a repair-competent Chinese hamster ovary (CHO) cell line which appears to be functionally heterozygous at the *aprt* locus (Thompson *et al.* 1979). It was provided by Dr. Larry Thompson, Lawrence Livermore National Laboratory, Livermore, Ca., with the help of Dr. Gordon Whitmore, Physics Division, Ontario Cancer Institute, Toronto, Ont.

K1 is another repair-competent CHO cell line and was provided by Dr. Miria Stefanini, Istituto di Genetica Biochimica ed Evoluzionistica del C.N.R., Pavia, Italy.

A.2 UV-Sensitive DNA Repair Deficient Rodent Cell Lines

UV20, **UV5**, **UV24**, **UV41**, **UV135**, and **UV61** are members of the rodent excision repair complementation groups 1, 2, 3, 4, 5, and 6, respectively (Thompson *et al.* 1980; Busch *et al.* 1980). They have been isolated from the AA8 cell line and are deficient in the incision step of DNA nucleotide excision repair. These mutant cell lines were obtained from Dr. Larry Thompson with the help of Dr. Gordon Whitmore.

5T4-12 is a UV5 cell line which has been stably transfected with a genomic clone of the human repair gene *ERCC2* (Weber *et al.* 1988).

5-2ER2-6 has been derived by transformation of a UV5 cell line with an *ERCC2* cDNA expression plasmid (Weber *et al.* 1991).

5T4-12 and 5-2ER2-6 were provided by Dr. Christine Weber, Lawrence Livermore National Laboratory, Livermore, Ca.

30PV, **50PV**, **7PV**, and **4PV** belong to rodent excision repair complementation groups 1, 5, 9, and 10, respectively (Stefanini *et al.* 1989; Stefanini *et al.* 1991). These mutants were provided by Dr. Miria Stefanini.

A.3 Other Rodent Cells

EM9 was isolated from AA8 on the basis of its increased sensitivity to ethyl methanesulphonate (EMS) (Thompson *et al.* 1980). It was provided by Dr. Larry Thompson with the help of Dr. Gordon Whitmore.

UCL was originally thought to be EM7-2, a mutant derived from AA8 on the basis of its sensitivity to ethane methylsulfonate (Thompson *et al.* 1980).

A cell sensitivity assay was performed for a number of cell lines including UCL, which was originally thought to be EM7-2 (Figure 4). This assay raised questions as to the identity of the UCL cell line, thus its designation. This matter is examined further in the discussion.

Figure 4: UV Cell Sensitivity Assay for AA8, UV20, EM9, UCL, UV5, 5T4-12

Cells were seeded at 1.0 x 10^5 per well in 24 well dishes and allowed to settle and adhere to the dish. Medium was then aspirated and replaced with 0.2 mL of pre-warmed PBS. Cells were irradiated with the noted UV fluences. The PBS was aspirated from the wells and the cells were overlayed with fresh pre-warmed growth medium. Several days later, the wells were stained with crystal violet.

UV Fluence
to Cells
(J/m2)
0
1
2
4
8
UV20
UCL
EM9
AA8
UV20
ST4-12
UV5
AA8

A.4 Cells of Human Origin

293 cells are human embryonic kidney cells transformed with sheared Ad 5 DNA (Graham *et al.* 1977). These cells were provided by Dr. Frank Graham, Departments of Biology and Pathology, McMaster University, Hamiltion, Ontario.

The **HeLa** cell line (CCL2) was obtained from American Type Culture Collection (ATCC), 12 301 Parklawn Drive, Rockville, MD, USA.

The **HT29** cell line originated from a colon carcinoma and was supplied by Dr. William Orr, Department of Pathology, McMaster University, Hamiltion, Ontario.

XP1BR group D (GM03615) are human fibroblasts belonging to xeroderma pigmentosum, complementation group D. These were purchased from the NIGMS Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, 401 Haddon Ave., Camden, NJ, 08103.

GM969 cells are normal human diploid fibroblasts. These were purchased from the NIGMS Human Genetic Mutant Cell Repository.

A.5 Growth and Passaging of Cells

All cell lines were grown in α-MEM (Grand Island Biological Company (Gibco)-BRL Canada Inc., 2270 Industrial Street, Burlington, Ontario, L7P 1A1. Cat.#410-2000) which was purchased in powdered form, dissolved in glass double distilled water and subsequently filter sterilized using a 0.22 micron filter.

The media was supplemented with the following:

1. newborn calf serum (Gibco-BRL, Cat. #200-6010AJ) to a final concentration of 10% (vol/vol)

2. antibiotic-antimycotic (10 000 units/mL penicillin G sodium, 10 000 mg/mL streptomycin sulfate, and 25 mg/mL amphotericin B as Fungizone) (Gibco-BRL Cat.#600-5240AG) to a final concentration of 1% (vol/vol)

Cells were grown in 75 cm^2 screw-cap bottles (Corning Incorporated, Corning, New York. Cat.#25115-75) or 150 cm^2 plastic Nunc dishes (Nunclon Intermed, Denmark) in a humidified chamber at 37°C and 5% CO_2. When the cells became confluent, the medium was aspirated off and 2 to 4 mL of trypsin (Gibco-BRL, Cat.#610-5400AG), diluted to 2X in PBS, was added before returning the dish to the incubator. When the cells began to round up, usually within one to two minutes, the dish was rapped sharply to dislodge the cells from the plastic surface and an appropriate volume of medium was added to the dish to permit subculturing. Cells of human origin were generally subcultured at a ratio of 1 to 3 or 1 to 4 whereas the CHO cells were subcultured at ratios as high as 1 to 20.

B. VIRUSES

B.1 Viral Strains

Wild-type adenoviruses used in this study:

Human adenovirus type 2 (**Ad 2**)

Human adenovirus type 5 (**Ad 5**)

Other viruses used in this work include the following Ad 5 mutants:

Ad5(MTR3) contains an insertion of polyoma virus in the E1 region and was a gift from Dr. John A. Hassell, Institute of Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario. (Rainbow and Castillo (in press)).

Ad5(LacZ) contains β-galactosidase inserted into the deleted E3 region and was provided by Dr. Frank Graham, Departments of Biology and Pathology, McMaster University, Hamilton, Ontario.

Ad5(denV) contains the bacteriophage T4 pyrimidine dimer DNA glycosylase (*denV*) gene inserted into the deleted E3 region (Colicos *et al.* 1991)

Ad5(ERCC1) contains the1.1 kb cDNA of the human DNA repair gene, ERCC1, inserted in the deleted E3 region of the virus (Rainbow and Castillo 1992).

B.2 Preparation of Viral Stocks

293 cells, grown to confluency in 150 cm^2 dishes, were infected at a multiplicity of infection of 1-2 pfu/cell in 4 mL of α-MEM containing 1% antibiotic-antimycotic. After a period of 90 minutes, 16 mL of complete growth media was added to each dish. When complete cytopathic effect (CPE) was evident, usually 48 to 72 hours post-infection, scraping with rubber policemen and/or pipetting of the media up and down was done to ensure that all cells were detached from the dishes. The cell suspension was then transferred to 50 mL conical tubes (Corning, Corning New York, Cat.#25331-50) and centrifuged at 1000 rpm for 10 minutes to pellet the cells. The supernatant was then discarded and, for each 150 cm^2 dish, the cells were resuspended in 1-2 mL of a filter sterilized solution of 10% glycerol in α-MEM.

B.3 Titering of Viral Stocks

Normalization of various viral stocks was done by determining the concentration of plaque forming units (PFU) of various stocks as well as by compararison of the Ad genome equivalents of different stocks.

B.3.i Titering By Plaque Formation

Virus was released from the 293 cells into the 10% glycerol/a-MEM by freeze/thawing three times. A serial dilution of the viral stock was then performed in α-MEM and 1% antibiotic-antimycotic. Confluent monolayers of 293 cells in 60 mm dishes were infected with the various dilutions. After allowing 90 minutes for the virus to adsorb to the cells, an overlay of 10% NCS, 1% antibiotic-antimycotic, 0.2% yeast extract and 0.5% agarose in 2X F11 medium was applied to the monolayers. When the plaques were large enough to count, usually 6-8 days post-infection, the monolayers were fixed and stained with 1% crystal violet, 10% methanol, and 18% formaldehyde solution in 1X PBS. The number of plaques formed by each dilution was then used to calculate the titer of the viral stock.

B.3.ii Titering By Viral DNA Content

The amount of viral DNA present in various viral stocks was also expressed as Ad DNA genome equivalents and used as a measure of normalization between stocks. Viral DNA was extracted from small aliquots of the viral stocks, slot-blotted to GeneScreenPlus (Dupont Canada Inc., 2453 46th Ave., Lachine Quebec H8T 3C9, Cat.#NEF-976), hybridized to a radioactively labelled Ad specific probe, and counted by scintillation. The viral DNA content of these stocks was determined by comparison with a serial dilution of Ad DNA of known concentrations which were included on the slot-

blot. Viral DNA content was then compared to the plaque forming units (PFU), as determined by plaquing on 293 cells, in these viral stocks (Table 11).

C. Host Cell Reactivation Assay

C.1 Preparation of Cells

Cells were trypsinized and resuspended in a small volume of complete growth medium. A hemacytometer (American Optical Company, Buffalo, USA) was used to determine the number of cells present in the suspension. The wells were then seeded in 24 well Linbro plates (Flow Laboratories Inc., Hamden, CT) at approximately 2 x 10^5 cells per well . Following the seeding of the cells, the plates were returned to the incubator, usually for 12-16 hours, to allow the cells to settle and adhere to the bottom of the wells.

C.2 Irradiation of Virus

When appropriate, virus was irradiated prior to infection as previously described (Rainbow 1977). Briefly, viral stocks were diluted 5- to 100- fold in α-MEM supplemented with 1% antibiotic-antimycotic. Volumes of these diluted viral suspensions of no greater that 1.4 mL were placed in 35 mm plates and irradiated at dose rates of 1-5 joules/m^2/sec. During the irradiation of the virus, the 35 mm plates were on ice and the suspension was swirled constantly.

C.3 Infection of Cells

Aliquots from the appropriate viral stock were diluted in α-MEM and 1% antibiotic-antimycotic to allow a multiplicity of infection of 40 pfu/cell. Following aspiration of the media, 0.2 mL of the viral suspension was added to each well . After incubating at 37°C for a period of 90 minutes, during which the plates were gently rocked at 15 minute intervals, each well was overlayed with 1 mL of pre-warmed growth medium. At four to six hours after the overlay, the medium from each well was aspirated and replaced with 0.5 mL of fresh pre-warmed growth medium. This last step removes any virions from the well which have not adsorbed to the cells.

C.4 Harvesting of DNA Samples

C.4.i DNA To Be Slot-Blotted

At the appropriate time following infection, 0.2 mL of lysis solution (4 mg/mL Pronase, 40 mM Tris pH 8.0, 40 mM EDTA pH 8.0, 2.4% SDS) was added directly to the 0.5 mL of growth medium contained in each well. Following the addition of the lysis solution, the wells were placed at 37°C for a period of 1.5 to 3 hours. After this time, the contents of the wells were collected with an Eppendorf pipetman and transferred to microfuge tubes which were then placed on ice until DNA extraction.

C.4.ii DNA To Be Run On A Gel

The DNA was harvested as described above, except that following the Pronase incubation at 37°C, NaAcetate pH 7.0 was added to a final concentration of 1 M. This precipitated the high molecular weight cellular DNA (Hirt 1967). Following centrifugation, the cellular DNA pellet was discarded and the viral DNA in the supernatant was extracted.

C.5 DNA Extraction

One volume of phenol:chloroform was added to one volume of DNA sample. The mixture was vortexed, centrifuged, and the aqueous phase transferred to another microfuge tube containing one volume of chloroform. Following vortexing and centrifugation, the aqueous phase was added to two volumes of cold absolute ethanol. NaCl was also added to a final concentration of 0.2 M. The samples were then stored at -20°C for a minimum of one hour after which they were centrifuged at 13 000 rpm for 30 minutes to pellet the DNA. The supernatant was poured off and the DNA pellets dried. The dried DNA samples were resuspended in a volume of TE buffer (10 mM Tris, 1 mM EDTA) pH 8.0 (usually 40 µL) and stored at -20°C until needed.

C.6 Quantification of Viral DNA

The relative amounts of viral DNA were quantified by either densitometric scanning of viral DNA bands on autoradiograms or scintillation counting.

C.6.i Method 1 - Densitometric Scanning

Agarose Gel Electrophoresis of DNA Samples

Restriction volumes of viral DNA samples were 30 µL; with 3 µL of the appropriate 10X restriction enzyme buffer, 10 units of restriction enzyme, 0.5 µg of RNase A, and double distilled sterile water. After restriction, samples were placed at -20°C. After pouring the agarose gel (0.8%) and immersing it in 1X phosphate buffer, 5 µL of gel-loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol in water) was added to each restricted DNA sample. 33 µL of each sample was loaded onto the gel. Electrophoresis was carried out in the cold room (4°C) at 70 to 100 volts for 18 to 24 hours.

Southern Blotting and Autoradiography

Once the electrophoresis was complete, a photograph of the ethidium bromide stained gel was taken under UV light after which the DNA was Southern blotted to GeneScreenPlus (Dupont Cat.#NEF-976) (Southern 1975). A radioactively-labelled probe was prepared and hybridized to the membrane. After washing and drying of the membrane, it was exposed to X-ray film to produce an autoradiogram.

Densitometric Scanning

A series of 2-fold dilutions of Ad DNA were slot-blotted to GeneScreenPlus (Dupont Cat.# NEF-976) and hybridized with a radioactively labelled DNA probe. An autoradiogram of this slot blot was then made. A laser microdensitometer was used to scan the autoradiograms obtained from both the slot blot and the gel electrophoresed viral DNA and the area corresponding to the viral bands on the gel and the slot blot determined. A standard calibration curve of area to relative viral DNA for the slot blot was created and used to assess the relative viral DNA corresponding to the bands on the autoradiogram of the Southern transferred DNA.

C.6.ii Method 2

Slot-Blotting of DNA Samples

The DNA was slot-blotted to GeneScreenPlus (Dupont Cat.# NEF-976) according to the manufacturer's specifications. Briefly, the DNA sample was denatured in 0.25 N NaOH for 10 minutes, chilled on ice, and then diluted to the desired concentration in 0.125 N NaOH, 0.125X SSC and then slot-blotted onto the membrane. The membrane was then allowed to dry.

Autoradiography

A radioactively labelled probe was hybridized to the membrane. After washing and drying of the membrane, it was exposed to X-ray film to produce an autoradiogram.

Scintillation Counting

The autoradiogram was aligned with the membrane in order to locate the bands of DNA. The viral bands were cut from the membrane and the amount of DNA on each piece of membrane determined by scintillation counting.

C.7 Preparation of Radioactive DNA Probe

The preparation of radioactively labelled DNA probes was done in one of two fashions. Nick translation and Klenow labelling of the DNA were used to produce radioactively labelled DNA probes.

C.7.i Nick Translation

A commercially available Nick Translation Kit (Fisher Scientific, 1200 Denison Street, Unionville, Ontario, L3R 8G6; Cat. # U1001) was used to prepare probes in this manner. The protocol followed was that suggested by the manufacturer.

C.7.ii Klenow Labelling (Random Primers)

More frequently, the DNA was labelled via the Klenow labelling procedure. The protocol followed was similar to that described in Sambrook *et al.* (1989) with the exception that the components of the reaction mixture were mixed in a microfuge tube.

D. Protein Studies

D.1 Antibodies

The antibodies used in this study included:

Rabbit Ad2 Polyclonal (Rainbow and Howes 1982) was prepared by Margaret Howes.

M73 Monoclonal anti-E1a (Harlow *et al.* 1985) was obtained from Dr. Stanley Bayley, Department of Biology, McMaster University, Hamilton, Ontario.

a H2 19AF Monoclonal anti 72K DBP was obtained from Dr. Ludvik Prevec, Departments of Biology and Pathology, McMaster University, Hamilton, Ontario.

Rabbit Anti-T4 Endonuclease V Polyclonal was obtained commercially from Applied Genetics Inc. (Cat. # 550 ABD)

D.2 Preparation of Cells

Cells were trypsinized and resuspended in a small volume of α-MEM supplemented with 10% NCS and 1% antibiotic-antimycotic. A hemacytometer was then used to calculate the appropriate resuspension volume to allow the cells to be seeded at 2.2×10^6 cells per 60 mm dish. This cell concentration is 9.26×10^4 per mm^2 and is similar to that used in the HCR assay. Following the seeding of the cells, the plates were returned to the incubator for a minimum of six hours to allow the cells to settle and adhere to the surface of the dishes.

D.3 Infection Of The Cells

Aliquots from the appropriate viral stock were diluted in α-MEM and 1% antibiotic-antimycotic to allow a multiplicity of infection of 40 pfu/cell. Following aspiration of the media from the dishes, 0.2 mL of the viral suspension was added to 60 mm dish. After incubation at 37° C for a period of 90 minutes, during which the dishes were gently rocked at 10 minute intervals, each dish was overlayed with 5 mL of pre-warmed growth medium.

D.4 Radioactive Labelling of Proteins

At the appropriate time, the cells were scraped from the bottom of the dishes with cell scrapers. The suspension of cells in the medium was transferred to 10 mL conical tubes. The dishes were then washed with 5 mL of prewarmed 1X PBS and the buffer then transferred to the conical tubes as well. The conical tubes were centrifuged at 1000 rpm for 10 minutes. The cell pellet was washed twice with 1X PBS. After the second washing, the cell pellets were resuspended in 1 mL of 199 met- medium supplemented with 1% antibiotic-antimycotic. The cells were then returned to the original 60 mm dishes and incubated at 37°C for 30 minutes. After the 30 minute period, 0.5 mL of 199 met- medium containing 90 mCi of ^{35}S-methionine and 1% antibiotic-antimycotic was added to each dish and the dishes returned to incubate at 37°C for 2 hours.

D.5 Isolation of Proteins

After the labelling period, the cells were carefully transferred to 10 mL conical tubes and centrifuged at 1000 rpm for 10 minutes. The radioactive supernatant was taken off, the cell pellet resuspended in 2 mL of 1X PBS, and the mixture centrifuged for 10 minutes at 1000 rpm. The supernatant was again removed and the cell pellet resuspended in 2.1 mL of RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% Nadeoxycholate, 0.1% SDS, 50 mM Tris pH 8.0). Two protease inhibitors, aprotinin (1 µg/mL) and phenylmethylsulfonylfluoride (PMSF) (50 µg/mL), were added at this time. The extract was incubated on ice

for 20 minutes after which it was put through a 22 gauge needle 4 times and then centrifuged at 13 000 rpm for 30 minutes at 4°C to pellet the nuclei and cellular debris. The supernatant was divided into 4 aliquots of 475 µL and 2 aliquots of 20 µL; the larger aliquots destined for immunoprecipitation reactions and the smaller ones for non-immunoprecipitations.

D.6 Immunoprecipitation of Proteins

The protein samples were mixed with the appropriate antibody and rotated for a minimum of 2 hours at 4°C. The amount of antibody added for the various immunoprecipitations was 15 µL, 1 µL, 3 µL, and 20 µL for rabbit anti-Ad2 polyclonal, M73 anti-E1a monoclonal, α H2 19AF anti-72K DBP monoclonal, and rabbit anti-T4 Endonuclease V polyclonal (50 µg/mL stock), respectively. Following this period of incubation, 100 µL of a suspension of protein A sepharose beads (0.28 g/4 mL) was added to each immunoprecipitation reaction. This incubation was allowed to proceed for a minimum of 1.5 hours, also rotating at 4°C. The beads were pelleted and washed three times with RIPA buffer. The pelleting was accomplished by centrifuging at 13 000 rpm for 1 minute at 4°C. After the third wash, the proteins were resuspended in 60 µL of 2X sample buffer (50 mM Tris pH 7.2, 2% SDS, 5% β-mercaptoethanol, 10% glycerol, 0.1% bromophenol blue) and heated to 100°C for 5 minutes after which they were placed in ice for 5 minutes. The tubes were centrifuged at 13 000 rpm for 2 minutes to pellet the beads and 30 mL of the supernatant was loaded on the gel.

D.7 SDS Polyacrylamide Gel Electrophoresis

The protein samples were resolved on 9% acrylamide gels (9% acrylamide, 0.37 M Tris pH 8.8, 0.1% SDS, 0.1% glycerol; polymerized by the addition of 0.1% ammonium persulfate, 0.35 mL/mL N,N,N',N'-Temed). A 5.6% acrylamide stacking gel (5.6% acrylamide, 0.12 M Tris pH 6.8, 0.1% SDS, 0.05% glycerol; polymerized by the addition of 0.06% ammonium persulfate, 0.72 mL/mL N,N,N',N'-Temed) of approximately 3 cm was poured on top of the polymerized resolving gel. The gels were run in vertical gel boxes in Laemmli buffer (25 mM Tris, 250 mM glycine pH 8.3, 0.1% SDS) at a current of 10-15 mA until the leading dye front was run to the bottom of the gel. SDS-PAGE standards used on these gels were high range (Biorad Cat.#161-0303), low range (Biorad Cat.#161-0304) or pre-stained low range standards (Biorad Cat.#161-0305).

D.8 Processing of Gels

After electrophoresis was complete, the gel was stained with Coomassie Blue (0.05% Coomassie Brilliant Blue, 10% glacial acetic acid, 50% methanol, 40% dd H_20) for 30 minutes with gentle agitation at room temperature. Gels were subsequently destained in 7% glacial acetic acid and 5% methanol. The radioactive signal was enhanced by treating the gel with dimethyl sulfoxide (DMSO) - polyphenoloxide (PPO). The gel was put in 100% DMSO for 1 hour followed by a further incubation in DMSO-PPO for 2 hours. The gel was then

rinsed in distilled water for a half hour before drying. The drying of the gel was done at 80°C for 1 hour. The gel was then exposed to film.

E. UV Enhanced Reactivation Assay

The UVER assay was the same as the HCR assay with the following modifications. After seeding the cells and allowing them to settle and adhere to the wells, they were UV irradiated at the appropriate time prior to viral infection. Viral DNA was then harvested, extracted, and quantified as described in the HCR assay.

E.1 Irradiation of Cells

UV irradiation of cells prior to infection was done as follows. The medium from the wells was aspirated and replaced with 0.2 mL of pre-warmed 1X PBS. The wells were then irradiated with a UV lamp (General Electric Germicidal Lamp G8T5) emitting a wavelength of predominantly 254 nm. The incident dose rate was 1-2 joules/m^2/sec as determined with a J-225 shortwave UV meter (Ultraviolet Products, San Gabriel, California). After irradiation, the 1X PBS was removed from each well and replaced with 0.5 mL of pre-warmed growth medium.

F. Analysis of Data

F.1 Surviving Fractions

In each HCR experiment, cells seeded in 24 well plates were infected with either irradiated virus or unirradiated virus. The amount of viral DNA synthesized in all cases was then quantified as described in the host cell reactivation assay. The viral DNA synthesis for each UV fluence was expressed as a fraction of that in unirradiated virus.

The averaged data points used for survival curves were obtained by averaging the log of the surviving fraction values for the same UV fluences from different experiments.

F.2 Do Values

Data points representing the log surviving fractions of viral DNA synthesis from all experiments were pooled. Linear regression analysis of these pooled points was used to obtain the slope of the best fit line along with the associated standard error. This slope was then used to calculate the D_0 value and its error expressed as the same percentage error as that associated with the slope of the best fit line.

RESULTS

A. Survival of Viral DNA Synthesis for UV-Irradiated Adenovirus In Mammalian Cells

Adenovirus (Ad) has been used extensively to probe the DNA repair capacity of permissively infected human cells (Rainbow 1981). In particular, the host cell reactivation (HCR) of Ad has been successfully applied in the detection of cellular DNA repair deficiencies (Rainbow 1981). HCR of UV-irradiated and sunlamp-irradiated Ad is reduced in XP and CS cells (Rainbow 1989a). HCR of UV-irradiated Ad is also reduced in human tumour cells and an SV40 transformed human cell line, suggesting an association of DNA repair deficiency with human tumourigenesis and the transformation of human cells (Rainbow 1989c). The Ad HCR assay has been successfully applied to human cells which are fully permissive to Ad infection and survival of the virus is scored by either plaque formation (Day 1974) or the formation of viral structural antigens (Vag) (Rainbow 1981). Ad infection of rodent cells is generally non-permissive or semi-permissive and does not give rise to plaque formation and viral reactivation in CHO cells has not been reported. However, in many semi-permissive infections by Ad 2, viral DNA synthesis takes place even though late viral protein synthesis is altered and no infectious virus is produced (Eggerding and Pierce; Longiaru and Horwitz 1981). In the work reported here, this property of semi-permissive Ad infections of CHO cells is used to examine the

ability of rodent cells to synthesize viral DNA after infection with UV-irradiated Ad.

Initial experiments investigated the time course of viral DNA synthesis in CHO cells infected with unirradiated Ad at a multiplicity of infection (MOI) of 40 PFU/cell. At various time points post-infection (p.i), the viral DNA was extracted from the cells and quantified by slot-blotting, hybridization with a radioactive Ad DNA probe and scintillation counting. The results of a typical experiment for CHO parental (AA8) and DNA repair-deficient mutants (UV20 and UV41) are displayed in Figure 5. This chart demonstrates that viral DNA synthesis in CHO cells begins to plateau approximately 48 hours p.i. and by 72 hours p.i. viral DNA synthesis has reached its maximum. Therefore, 72 hours p.i. was chosen as the time point at which viral DNA synthesis would be assessed in CHO cells for the HCR assay.

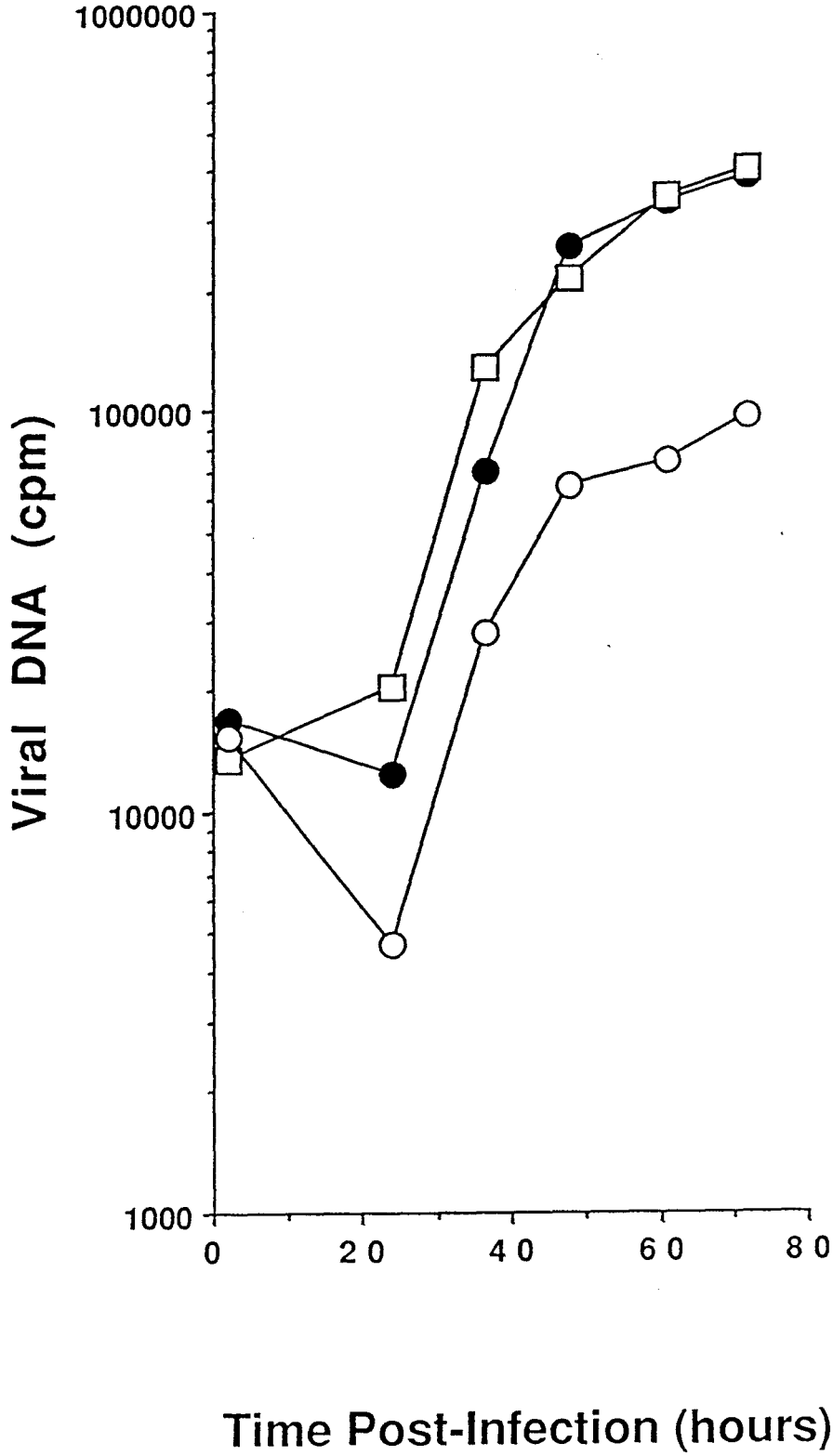
A.1. UV Survival of Ad5(LacZ) in Normal and DNA Repair Deficient CHO Cells

Since HCR of Ad has proven to be successful in the detection of DNA repair deficiencies in various human cells such as those from individuals with XP and CS, it was considered of interest to determine if HCR of Ad could be used to detect DNA repair deficiencies in CHO cells. Previous experiments in which UV-irradiated plasmids were transfected into DNA repair-deficient and DNA repair-proficient CHO cells produced conflicting results (Nairn *et al.* 1988). These workers were able to detect a deficiency in the frequency of transformants in CHO mutants when transfecting UV-irradiated plasmids

Figure 5: Viral DNA synthesis time course in CHO cells

The time course of viral DNA synthesis in CHO cells infected with wild-type adenovirus type 2 was examined. CHO cells were infected with Ad 2 at a MOI of 40 PFU/cell and the viral DNA extracted at the indicated times post-infection. The results of a typical experiment for a parental (AA8) and two UV-sensitive (UV20 and UV41) CHO cell lines are illustrated.

Closed circles	- AA8
Open circles	- UV20
Open squares	- UV41


1000000
100000
10000
1000
Viral DNA (cpm)
0
20
40
60
80
Time Post-Infection (hours)

containing the hamster adenine phosphoribosyltransferase (APRT) gene. However, the frequency of transformation in the CHO mutants was not significantly different from that of normal CHO when pSV2gpt was the UV-irradiated plasmid.

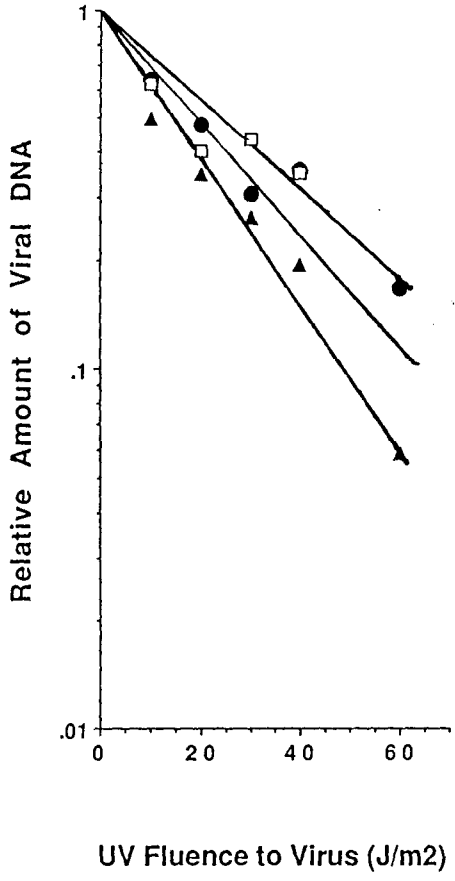
CHO cells were infected at a MOI of 40 PFU/cell with either UV-irradiated or unirradiated Ad5(LacZ) virus, which contains the β-galactosidase gene in the non-essential E3 region of the Ad genome. The Ad 5 mutant containing the β-galactosidase gene in the E3 region was deemed to be an appropriate control virus as the other recombinant viruses, Ad5(denV) and Ad5(ERCC1) contained inserts which similarly disrupted the E3 region of the Ad 5 genome. Furthermore, UV survival of Ad5(LacZ) DNA synthesis was seen to be the same as for wild type Ad 2 or for a co-infection of Ad 2 and Ad5(MTR3) (data not shown).

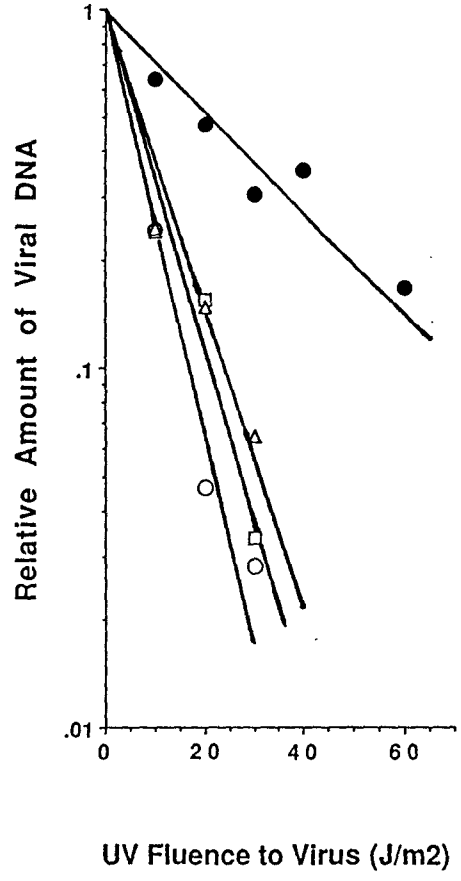
In each experiment, which included a parental cell line and at least two mutant cell lines, cells were infected with either UV-irradiated or non-irradiated Ad5(LacZ) and at 4 hours and 72 hours p.i., the viral DNA was extracted from the cells and quantified by slot-blotting, hybridization to a radioactive probe and scintillation counting. The net viral DNA synthesis was determined by subtracting the input viral DNA (4 hour p.i.) from the amount determined at 72 hours p.i. In individual experiments, the net viral DNA produced for each UV fluence to the virus was expressed as a fraction of that produced in cells infected with unirradiated virus. The pooled results from all experiments were then used to calculate the logarithmic mean of viral DNA survival for each UV fluence to the virus. The results for AA8, its derived UV-sensitive cell lines, and *ERCC2*-transfected UV5 cell lines are presented in Figure 6. The curves for

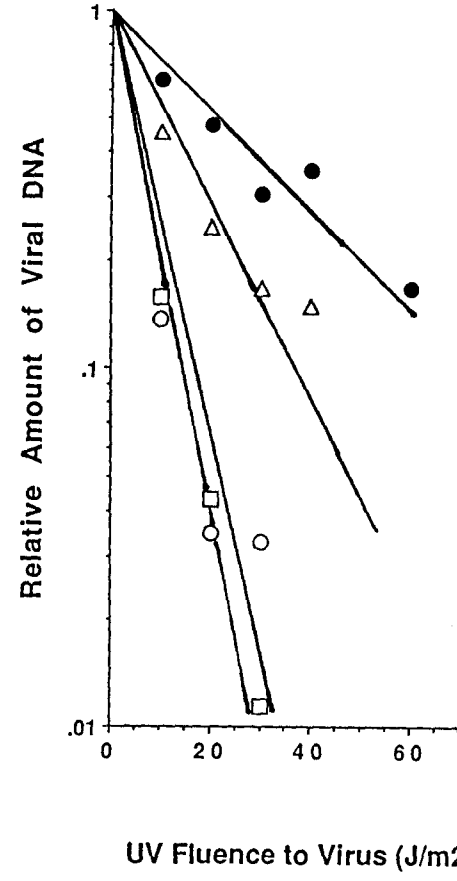
Figure 6: Survival of viral DNA synthesis in AA8 and its derived UV-sensitive cells infected with UV-irradiated Ad5(LacZ)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. The logarithmic means of surviving fractions of viral DNA synthesis for each UV fluence from all experiments were calculated, converted to mean surviving fractions and presented in this figure. The number of experiments which were pooled for each cell line are as listed in Table 2. Curves were fitted by eye.

Left panel:	Closed circles	- AA8
	Closed triangles	- 5T4-12
	Open squares	- 5-2ER2-6
Middle panel:	Closed circles	- AA8
	Open circles	- UV24
	Open squares	- UV41
	Open triangles	- UV135
Right Panel:	Closed circles	- AA8
	Open circles	- UV20
	Open squares	- UV5
	Open triangles	- UV61


Relative Amount of Viral DNA
1
.1
.01
0
20
40
60
UV Fluence to Virus (J/m2)


Relative Amount of Viral DNA
1
.1
.01
0
20
40
60
UV Fluence to Virus (J/m2)


Relative Amount of Viral DNA
1
.1
.01
0
20
40
60
UV Fluence to Virus (J/m2)

UV20, UV5, UV24, UV41, and UV135 demonstrate deficiencies in the UV survival of viral DNA synthesis compared to AA8. The UV61 cell line is seen to have a less pronounced reduction while 5-2ER2-6 is comparable to AA8 and 5T4-12 exhibits a small but significant reduction.

Linear regression analysis of the pooled data from all experiments for each cell line was used to calculate the D_0 values for UV survival of viral DNA synthesis of each cell line. These D_0 values, along with their associated standard errors, are presented in histogram form (Figure 7) and are summarized in Table 1. These results indicate that mutants from groups 1 through 5 have HCR values of 18.8 $\pm$ 4.8% to 30.8 $\pm$ 9.0% of parental AA8 (Table A). The % HCR value for group 6 member UV61 is 48.0 $\pm$ 14.6%. The genomic *ERCC2*-transfected 5T4-12 and *ERCC2* cDNA-transfected 5-2ER2-6 exhibit % HCR values of 60.0 $\pm$ 12.6% and 169.7 $\pm$ 95.3%, respectively (Table 3). Thus, HCR of UV-irradiated Ad5(LacZ) is significantly reduced in 5T4-12 while 5-2ER2-6 is not detectably different compared to AA8.

The D_0 values obtained from pooled data for EM9 and UCL (Figure 8; Table 1; Table 3) indicate a HCR value of 184.5 $\pm$ 49.7% for EM9 and a value of 23.5 $\pm$ 5.0% for UCL with respect to AA8.

Another CHO parental cell line, K1, and its derived UV-sensitive mutants were analyzed for UV survival of Ad5(LacZ) DNA synthesis as well (Figure 9). The data obtained for K1 and its mutants was subject to more variation than the data obtained for AA8 and its derived cell lines. This is reflected in the errors associated with the D_0 values and %HCR values calculated for K1 and its mutants. Linear regression analysis of pooled data from all experiments was used to determine D_0 values and the results are summarized in Table 2. 30PV

Figure 7: Histogram of D_0 values for viral DNA synthesis of AA8 and its derived UV-sensitive cells following UV fluence to Ad5(LacZ)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. Linear regression analysis of pooled logarithmic surviving fractions of viral DNA synthesis from all experiments, as listed in Table 2, was used to determine D_0 values. Error bars represent standard error.

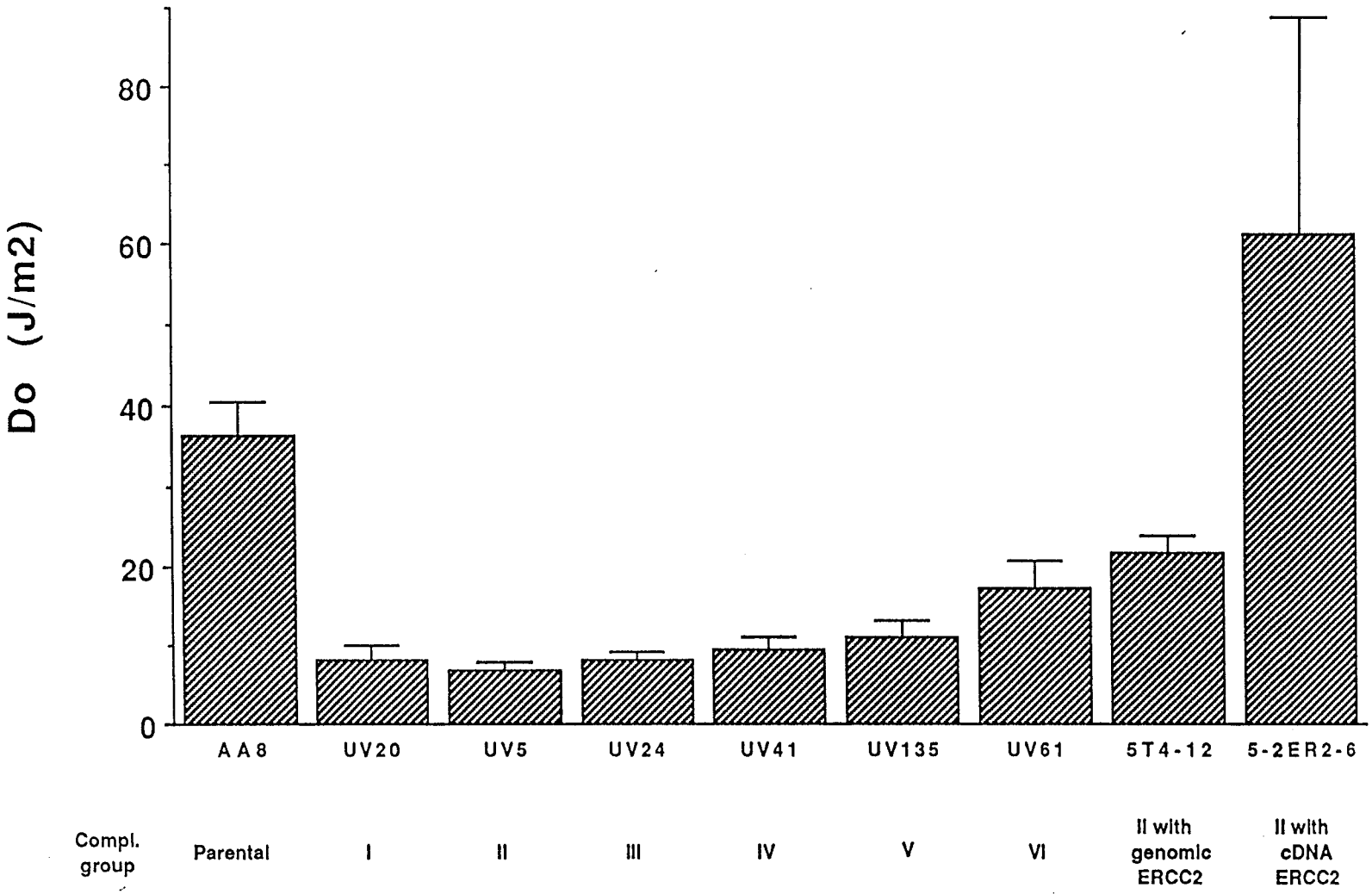

Do (J/m2)
80
60
40
20
0
AA8
UV20
UV5
UV24
UV41
UV135
UV61
5T4-12
5-2ER2-6
Compl. group
Parental
I
II
III
IV
V
VI
II with genomic ERCC2
II with cDNA ERCC2

Figure 8: Histogram of D_0 values for viral DNA synthesis of AA8 and derived cells following UV fluence to Ad5(LacZ)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. Linear regression analysis of pooled logarithmic surviving fractions of viral DNA synthesis from all experiments, as listed in Table 2, was used to determine D_0 values. Error bars represent standard error.

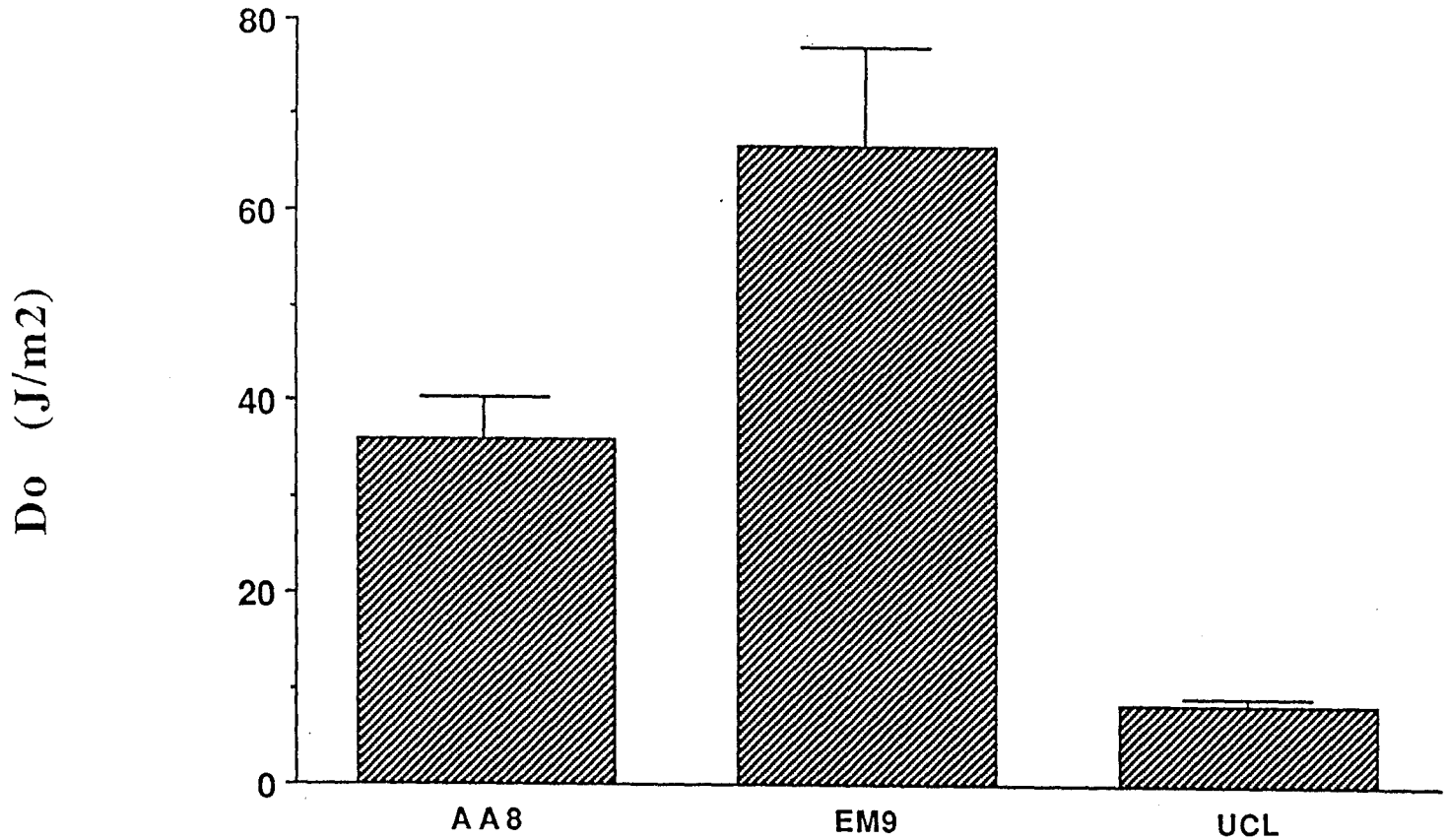
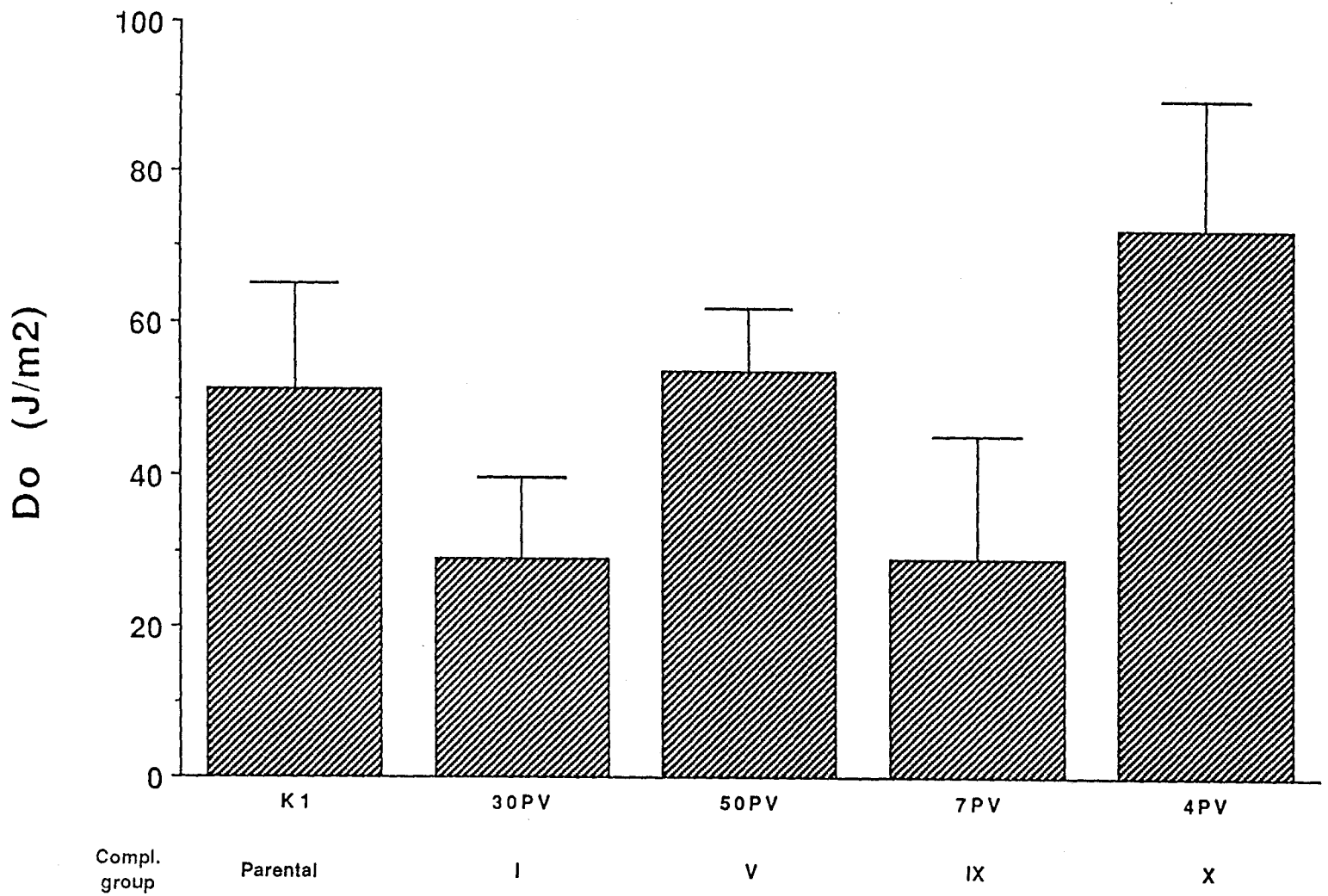

Do (J/m2)
80
60
40
20
0
AA8
EM9
UCL

Figure 9: Histogram of D_0 values for viral DNA synthesis of K1 and its derived UV-sensitive cells following UV fluence to Ad5(LacZ)

In each experiment, which included K1 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in K1 cells and its derived DNA repair mutants. Linear regression analysis of pooled logarithmic surviving fractions of viral DNA synthesis from all experiments, as listed in Table 4, was used to determine D_0 values. Error bars represent standard error.


Do (J/m2)
100
80
60
40
20
0
K1
30PV
50PV
7PV
4PV
Compl. group
Parental
I
V
IX
X

is the only PV mutant which demonstrates a significant reduction in %HCR of Ad5(LacZ) DNA synthesis with a value of 56.5 ± 36.2% (Table 3). The errors associated with the HCR values of the PV mutants are large and render the identification of deficiencies, particularly smaller ones, very difficult.

The HCR of UV-irradiated Ad(LacZ) in CHO cells has identified repair deficiencies of varying degrees in UV20, UV5, UV24, UV41, UV135, UV61, 5T4-12, UCL, and 30PV. Deficiencies were not detected in 5-2ER2-6, EM9, 50PV, 7PV, or 4PV.

A.2. UV Survival of Ad5(denV) in Normal and DNA Repair Deficient CHO Cells

The *denV* gene, under control of the Rous Sarcoma Virus LTR promoter and the SV40 poly signal sequence, has been inserted into the deleted E3 region of the Ad genome (Colicos *et al.* 1991). Colicos *et al.* (1991) demonstrated an increased UV survival of Ad5(denV) compared to control virus in fibroblasts from individuals with XP groups A, C, and E although this increased survival did not attain levels seen in normal fibroblasts. It was therefore considered of interest to examine the ability of Ad5(denV) to increase survival of viral DNA synthesis in CHO cells.

In individual experiments, which included a parental cell line and at least two mutant cell lines, cells were infected with either UV-irradiated or non-irradiated Ad5(denV) and the viral DNA was extracted from the cells at 4 hours and 72 hours p.i. Viral DNA was then quantified by slot-blotting, hybridization to

a radioactive probe and scintillation counting. The net viral DNA synthesized at 72 hours was obtained by subtracting the 4 hour viral DNA value from the 72 hour viral DNA value. In individual experiments, the net viral DNA produced for each UV fluence to the virus was expressed as a fraction of that produced in cells infected with unirradiated virus. The pooled results from a number of experiments, as indicated in Table 1, were then used to calculate the logarithmic mean of viral DNA survival for each UV fluence to the virus. The survival curves of Ad5(denV) DNA synthesis thus generated for AA8 and three of its derived cell lines are depicted in Figure 10. This figure indicates that the UV survival of viral DNA synthesis of Ad5(denV) is greater than that of Ad5(LacZ) for the parental AA8, the genomic*ERCC2*-transfected 5T4-12, and the DNA repair-deficient UV5 and UV135. Figure 12 demonstrates the increased D_0 values for UV survival of Ad5(denV) DNA synthesis compared to that for Ad5(LacZ) for AA8 and all its derived UV-sensitive mutants.

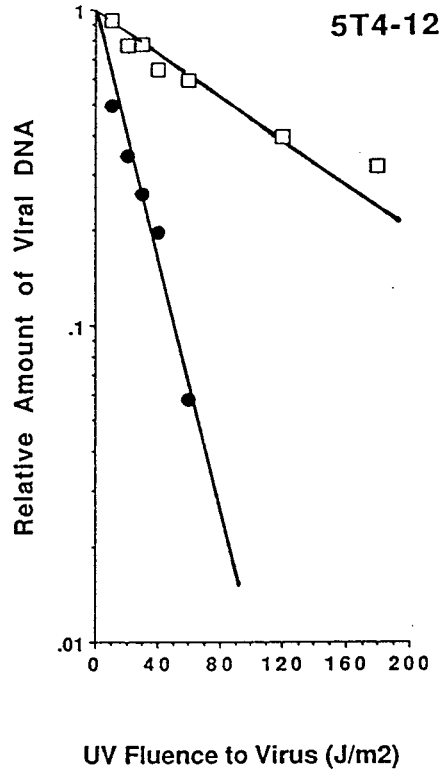
An increase in the UV survival of Ad5(denV) DNA synthesis compared to that of Ad5(LacZ) is evident in EM9 (Figure 13). Interestingly, UCL does not exhibit the increase in UV survival of Ad5(denV) DNA synthesis that is found in AA8 and its other derived cell lines. The D_0 values calculated from linear regression analysis of pooled data are listed in Table 1.

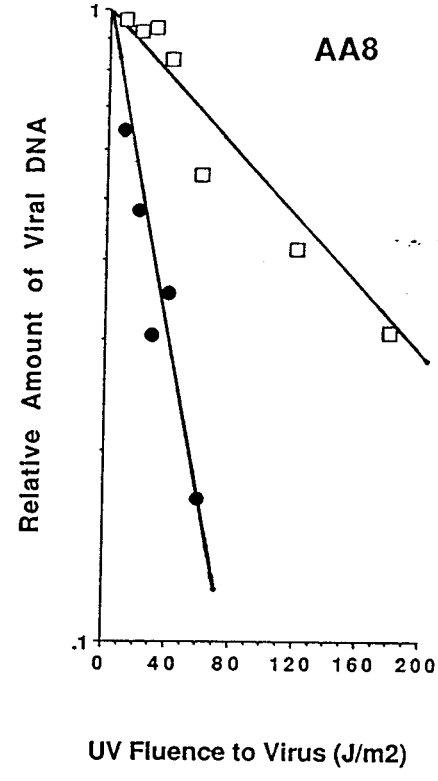
The ratios of D_0 for Ad5(denV) to Ad5(LacZ), along with the sum of the percentage errors of these two values, for AA8 and all its derived mutants are summarized in Table 5. With the exception of 5-2ER2-6 and UCL, Ad5(denV) significantly increases the UV survival of viral DNA synthesis in all these cell lines compared to Ad5(LacZ). UV survival of Ad5(denV) is enhanced to the greatest extent in 5T4-12. Its Ad5(denV) to Ad5(LacZ) D_0 ratio is 6.96 ± 1.25.

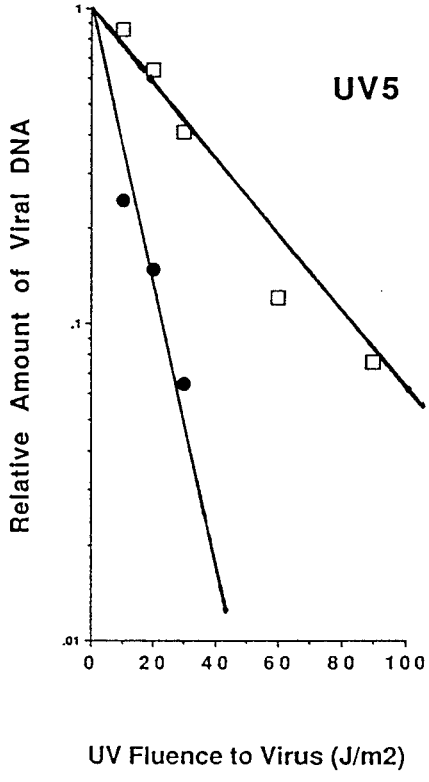
Figure 10: Survival of viral DNA synthesis in AA8 and its derived UV-sensitive cells infected with UV-irradiated Ad5(denV)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(denV) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. The logarithmic means of surviving fractions of viral DNA synthesis for each UV fluence from all experiments were calculated, converted to mean surviving fractions and presented in this figure. The number of experiments which were pooled for each cell line are as listed in Table 2. Ad5(LacZ) curves were included for comparison. Curves were fitted by eye.

Upper left panel:	Closed circles	- AA8, Ad5(LacZ)
	Open squares	- AA8, Ad5(denV)
Upper right panel:	Closed circles	- 5T4-12, Ad5(LacZ)
	Open squares	- 5T4-12, Ad5(denV)
Lower left panel:	Closed circles	- UV5, Ad5(LacZ)
	Open squares	- UV5, Ad5(denV)
Lower right panel:	Closed circles	- UV135, Ad5(LacZ)
	Open squares	- UV135, Ad5(denV)


5T4-12
Relative Amount of Viral DNA
1
.1
.01
0
40
80
120
160
200
UV Fluence to Virus (J/m2)


AA8
Relative Amount of Viral DNA
1
.1
0
40
80
120
160
200
UV Fluence to Virus (J/m2)


UV5
Relative Amount of Viral DNA
1
.1
.01
0
20
40
60
80
100
UV Fluence to Virus (J/m2)

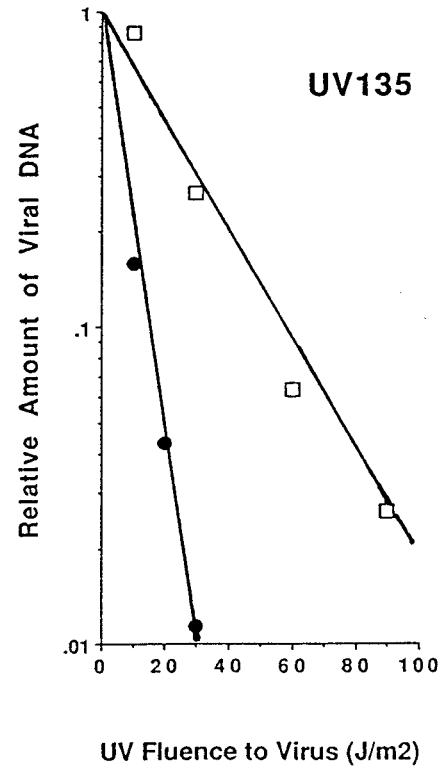
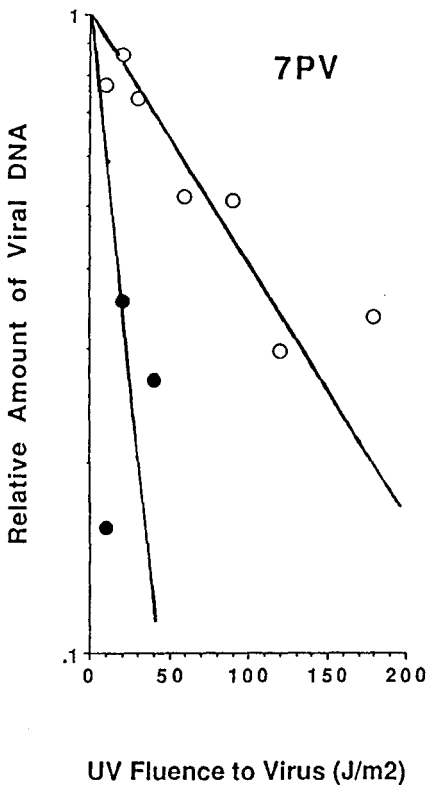
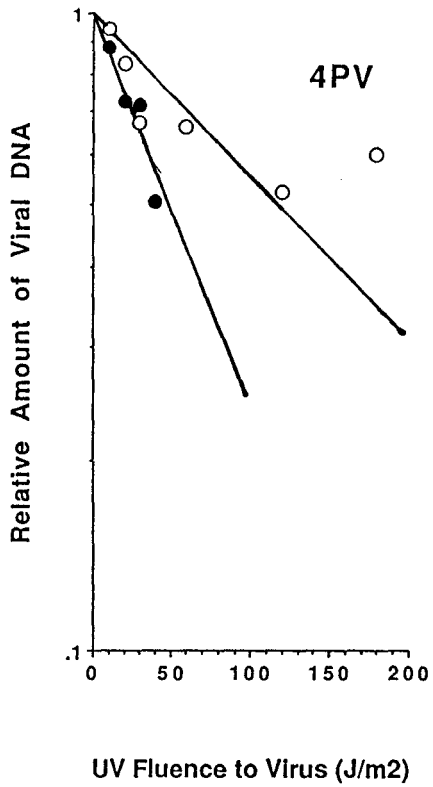

UV135
Relative Amount of Viral DNA
1
.1
.01
0
20
40
60
80
100
UV Fluence to Virus (J/m2)

Figure 11: Survival of viral DNA synthesis in K1-derived UV-sensitive cells infected with UV-irradiated Ad5(denV)

In each experiment, which included K1 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(denV) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in K1 cells and its derived DNA repair mutants. The logarithmic means of surviving fractions of viral DNA synthesis for each UV fluence from all experiments were calculated, converted to mean surviving fractions and presented in this figure. The number of experiments which were pooled for each cell line are as listed in Table 4. Ad5(LacZ) curves were included for comparison. Curves were fitted by eye.

Left panel:	Closed circles	- 7PV, Ad5(LacZ)
	Open circles	- 7PV, Ad5(denV)
Middle panel:	Closed circles	- 4PV, Ad5(LacZ)
	Open circles	- 4PV, Ad5(denV)
Right panel:	Closed circles	- 30PV, Ad5(LacZ)
	Open circles	- 30PV, Ad5(denV)


7PV
Relative Amount of Viral DNA
1
.1
0
50
100
150
200
UV Fluence to Virus (J/m2)


4PV
Relative Amount of Viral DNA
1
.1
0
50
100
150
200
UV Fluence to Virus (J/m2)

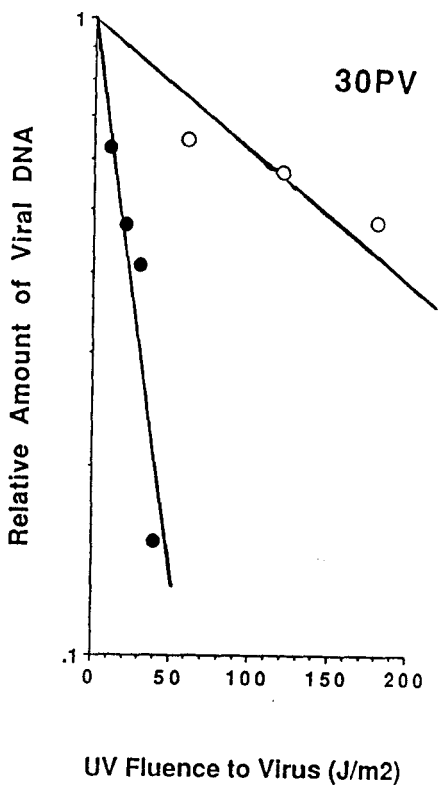

30PV
Relative Amount of Viral DNA
1
.1
0
50
100
150
200
UV Fluence to Virus (J/m2)

Figure 12: Histogram of D_0 values for viral DNA synthesis of AA8 and its derived UV-sensitive cells following UV fluence to Ad5(denV)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(denV) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. Linear regression analysis of pooled logarithmic surviving fractions of viral DNA synthesis from all experiments, as listed in Table 2, was used to determine D_0 values. Error bars represent standard error. Values for Ad5(LacZ) have been included for comparison.

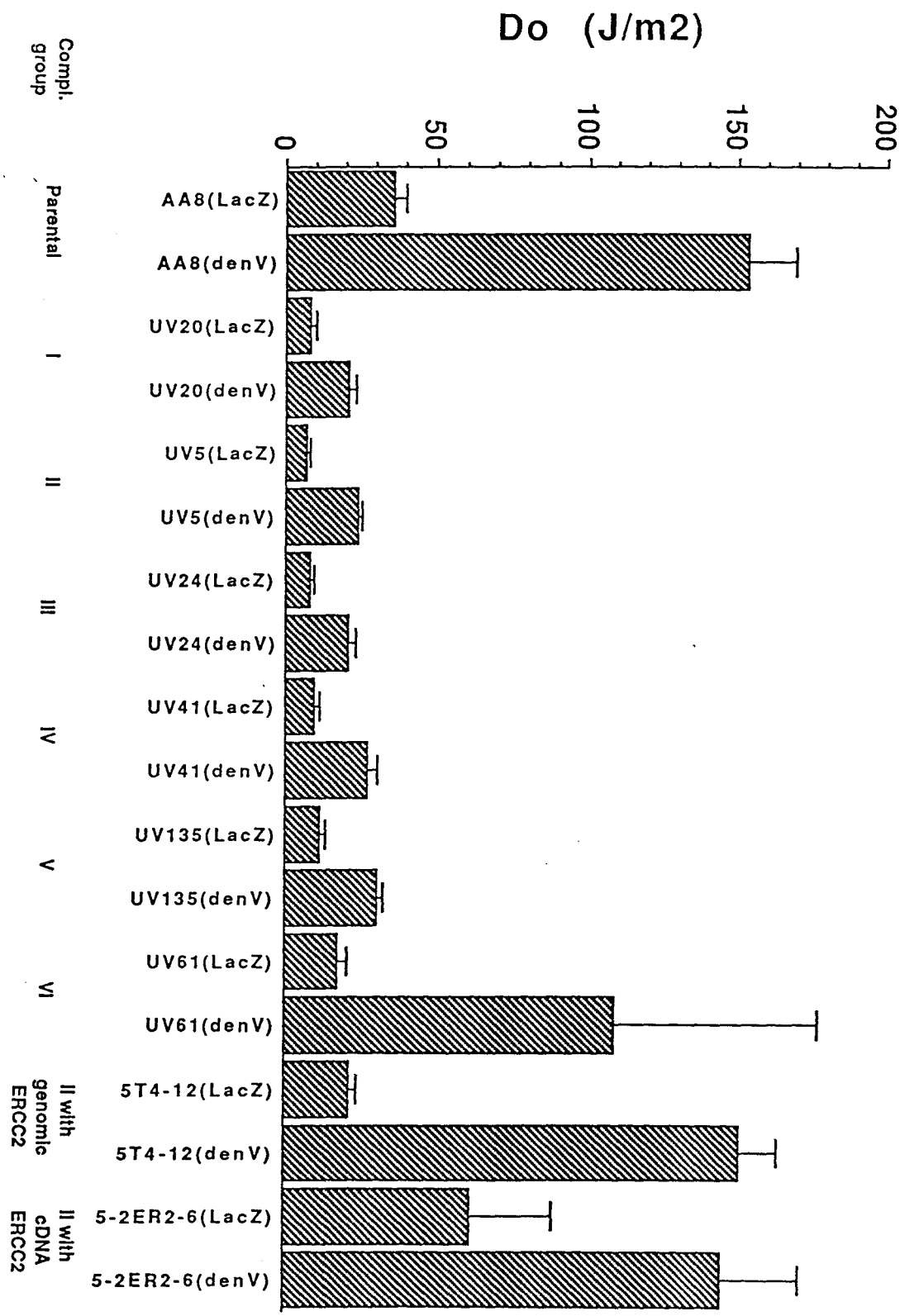
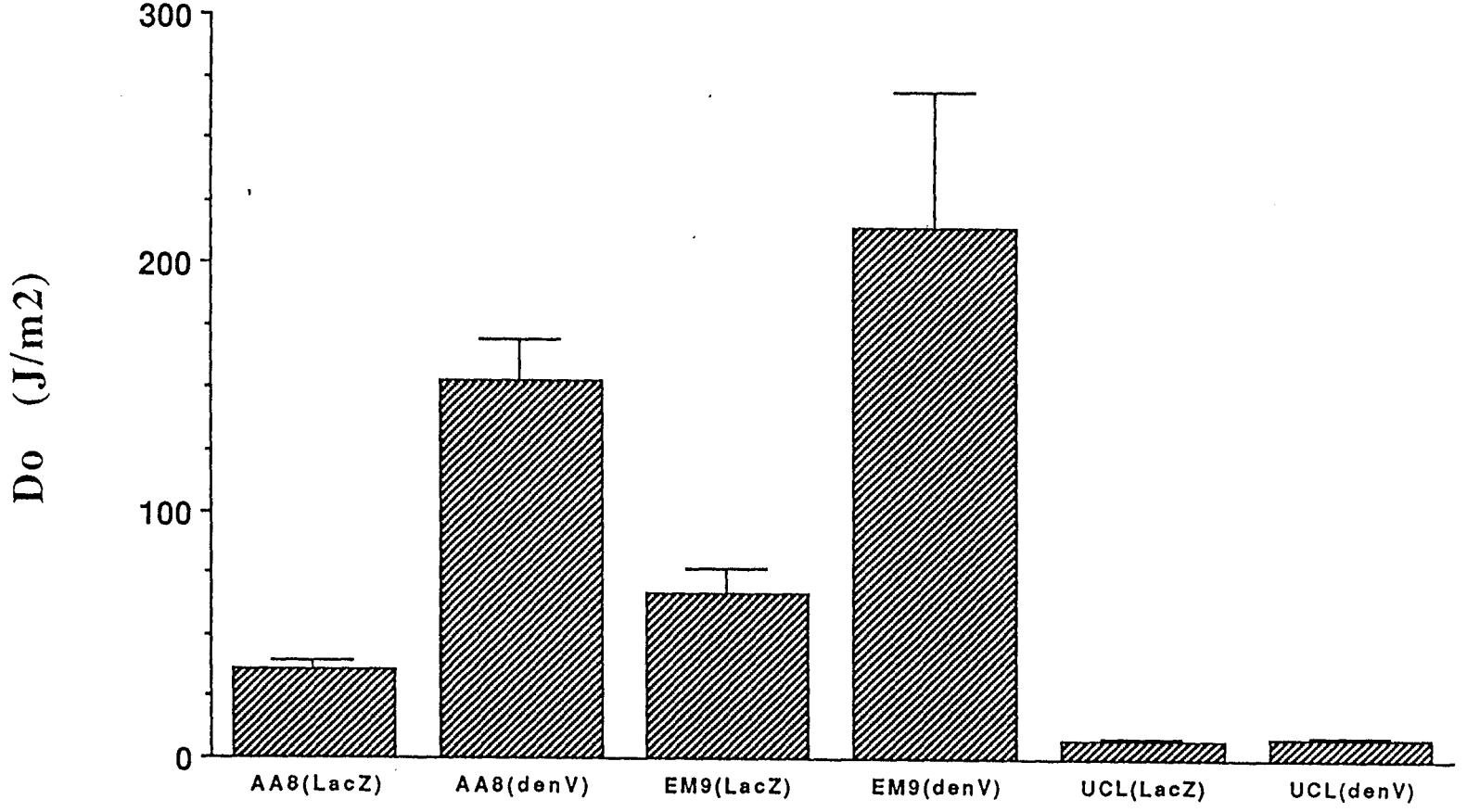

Do (J/m2)
0
50
100
150
200
Compl. group
Parental
I
II
III
IV
V
VI
II with genomic ERCC2
II with cDNA ERCC2
AA8(LacZ)
AA8(denV)
UV20(LacZ)
UV20(denV)
UV5(LacZ)
UV5(denV)
UV24(LacZ)
UV24(denV)
UV41(LacZ)
UV41(denV)
UV135(LacZ)
UV135(denV)
UV61(LacZ)
UV61(denV)
5T4-12(LacZ)
5T4-12(denV)
5-2ER2-6(LacZ)
5-2ER2-6(denV)

Figure 13: Histogram of D_0 values for viral DNA synthesis of AA8 and its derived cells following UV fluence to Ad5(denV)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(denV) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. Linear regression analysis of pooled logarithmic surviving fractions of viral DNA synthesis from all experiments, as listed in Table 2, was used to determine D_0 values. Error bars represent standard error. Values for Ad5(LacZ) have been included for comparison.


Do (J/m2)
300
200
100
0
AA8(LacZ)
AA8(denV)
EM9(LacZ)
EM9(denV)
UCL(LacZ)
UCL(denV)

The increases in UV survival of Ad5(denV) DNA synthesis of the other lines are approximately 2 to 4 fold and are all significantly less than that for 5T4-12.

An analysis of the survival curves derived for 7PV, 4PV, and 30PV demonstrate increases in survival for Ad5(denV) DNA synthesis compared to that for Ad5(LacZ) in each of these cell lines (Figure 11). The D_0 values for Ad5(denV) DNA synthesis calculated from linear regression of the pooled data from all experiments for K1, 30PV, 50PV, 7PV, and 4PV are enumerated in Table 2. This data reveals a significant increase in UV survival of Ad5(denV) DNA synthesis compared to that of Ad5(LacZ) for K1 and all its derived UV-sensitive mutants investigated (Figure 14). A determination of the ratio D_0 - Ad5(denV) to D_0 - Ad5(LacZ) demonstrates that UV survival of Ad DNA synthesis is enhanced to a lesser extent in 4PV than the other PV mutants although 4PV does not differ significantly from the parental K1 (Table 6).

A.3. UV Survival of Ad5(ERCC1) in Normal and DNA Repair Deficient CHO Cells

The construction of an Ad vector containing a cDNA for human *ERCC1* in the non-essential E3 region of the viral genome is the first report of a viral vector encoding a human DNA repair protein (Rainbow and Castillo 1992). The *ERCC1* cDNA insert is promoterless and as such transcriptional control of *ERCC1* is thought to be under control of Ad promoters.

The ERCC1 protein, which bears significant homology to the yeast RAD10 excision repair protein, contains several DNA binding domains (van Duin *et al.* 1986). It has been shown to be specific in its correction of the UV-

sensitivity of CHO mutants from complementation group 1, but not for that of mutant lines belonging to complementation groups 2 through 6 (van Duin *et al.* 1988a). It was considered to be of interest to examine the UV survival of Ad5(ERCC1) DNA synthesis in CHO parental, CHO mutants from complementation groups 9 and 10 as well as in cell lines from complementation groups 1 through 6.

Each experiment performed included a parental cell line and at least two mutants cell lines. Cells were infected with either UV-irradiated or non-irradiated Ad5(ERCC1) and viral DNA extracted from the cells at 4 hours p.i. and 72 hours p.i. Quantification of viral DNA was done by slot-blotting, hybridization to a radioactive Ad DNA probe and scintillation counting. The net amount of viral DNA synthesized by 72 hours p.i. was determined by subtracting the amount of viral DNA at 4 hours p.i. from the quantity found at 72 hours p.i. In individual experiments, the net viral DNA produced for each UV fluence to the virus was expressed as a fraction of that produced in cells infected with unirradiated virus. The pooled results from a number of experiments, as indicated in Table 1, were then used to calculate the logarithmic mean of viral DNA survival for each UV fluence to the virus. The survival curves of Ad5(ERCC1) DNA synthesis thus generated for AA8 and three of its derived cell lines are illustrated in Figure 15. This figure indicates that the UV-survival of Ad5(ERCC1) in UV20 is significantly increased in complementation group 1 UV20 cells as well as in parental AA8 cells but not in UV24 or UV41. Linear regression analysis of pooled data for all experiments, as listed in Table 1, was used to calculate the D_0 values for UV survival of Ad5(ERCC1) of AA8 and all its UV-sensitive mutants which are illustrated in Figure 17. Similarly, D_0 values for

Figure 14: Histogram of D_0 values for viral DNA synthesis of K1 and its derived UV-sensitive cells following UV fluence to Ad5(denV)

In each experiment, which included K1 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(denV) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in K1 cells and its derived DNA repair mutants. Linear regression analysis of pooled logarithmic surviving fractions of viral DNA synthesis from all experiments, as listed in Table 4, was used to determine D_0 values. Error bars represent standard error. Values for Ad5(LacZ) have been included for comparison.

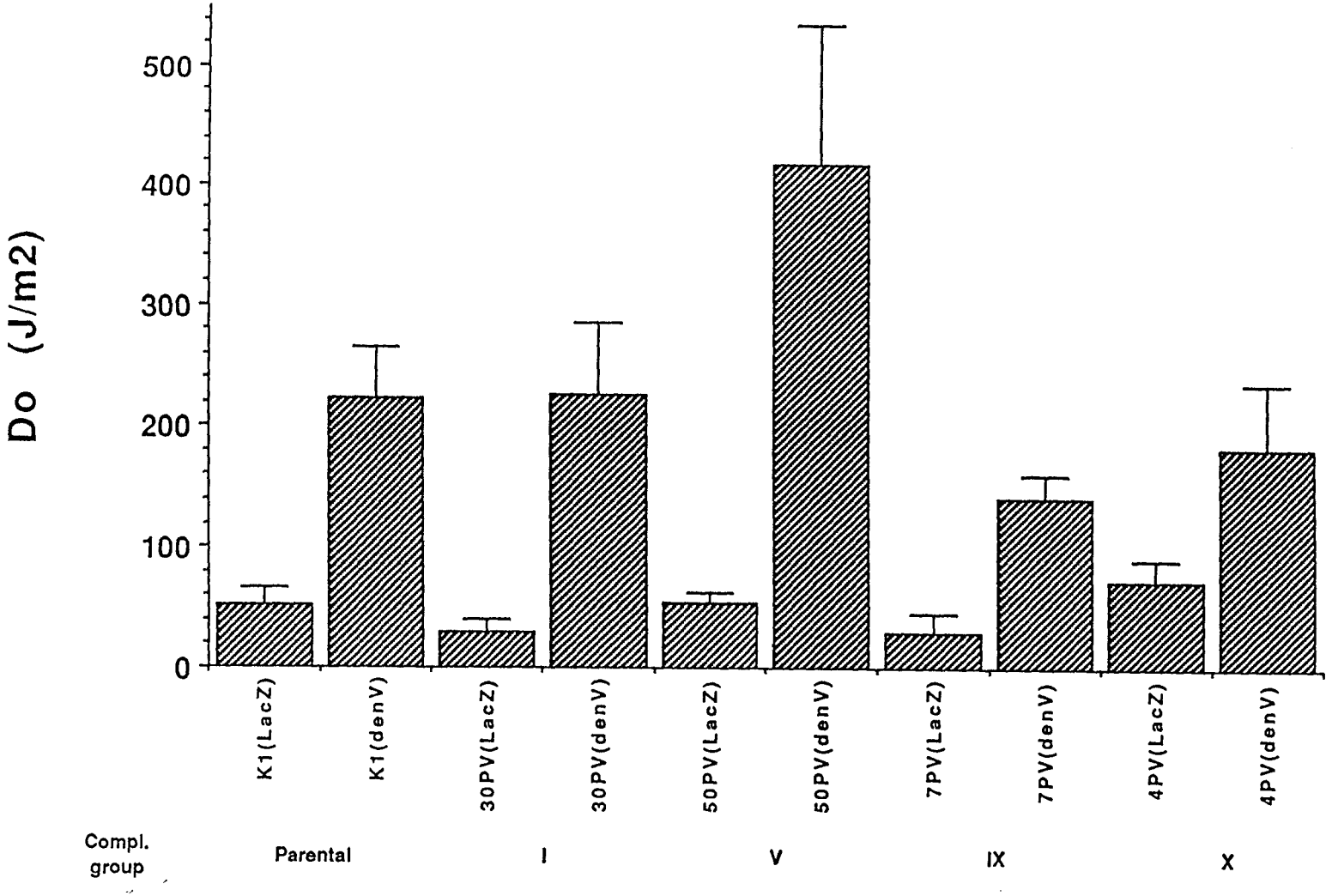
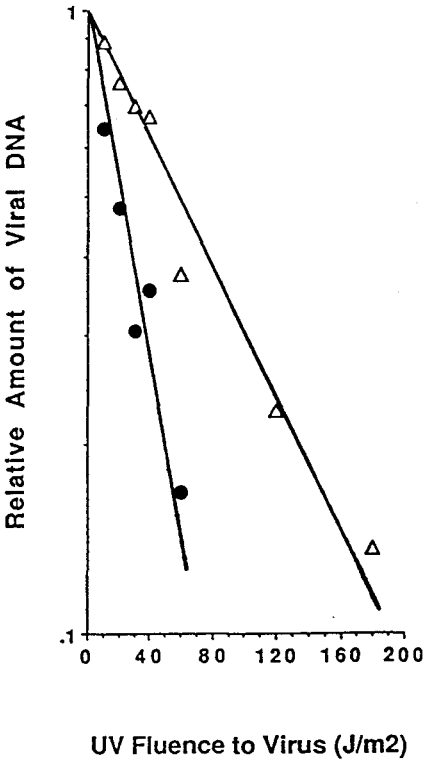
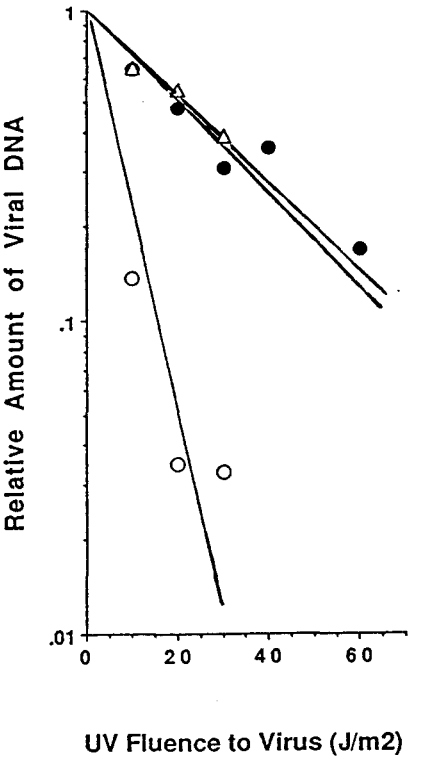

Do (J/m2)
500
400
300
200
100
0
K1(LacZ)
K1(denV)
30PV(LacZ)
30PV(denV)
50PV(LacZ)
50PV(denV)
7PV(LacZ)
7PV(denV)
4PV(LacZ)
4PV(denV)
Compl. group
Parental
I
V
IX
X

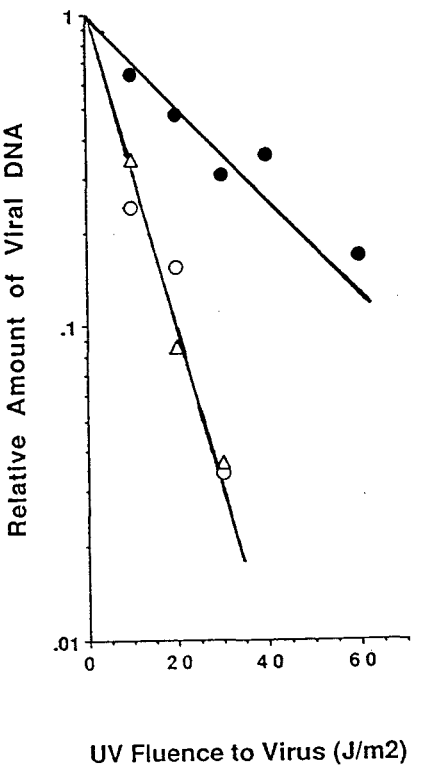
Figure 15: Survival of viral DNA synthesis in AA8 and its derived UV-sensitive cells following infection with UV-irradiated Ad5(ERCC1)

Each experiment included AA8 and at least two mutants which were infected with UV-irradiated or non-irradiated Ad5(ERCC1) at a MOI of 40 PFU/cell. Viral DNA synthesis was then scored at 72 hours p.i. The log means of surviving fractions of viral DNA synthesis for each UV fluence from a number of experiments, as listed in Table 2, were calculated, converted to mean surviving fractions and presented in this figure. Ad5(LacZ) curves were included for comparison. Curves were fitted by eye.

Upper left panel:	Closed circles	- AA8, Ad5(LacZ)
	Open triangles	- AA8, Ad5(ERCC1)
Upper right panel:	Closed circles	- AA8, Ad5(LacZ)
	Open circles	- UV20, Ad5(LacZ)
	Open triangles	- UV20, Ad5(ERCC1)
Lower left panel:	Closed circles	- AA8, Ad5(LacZ)
	Open circles	- UV41, Ad5(LacZ)
	Open triangles	- UV41, Ad5(ERCC1)
Lower right panel:	Closed circles	- AA8, Ad5(LacZ)
	Open circles	- UV24, Ad5(LacZ)
	Open triangles	- UV24, Ad5(ERCC1)


Relative Amount of Viral DNA
1
.1
0
40
80
120
160
200
UV Fluence to Virus (J/m2)


Relative Amount of Viral DNA
1
.1
.01
0
20
40
60
UV Fluence to Virus (J/m2)


Relative Amount of Viral DNA
1
.1
.01
0
20
40
60
UV Fluence to Virus (J/m2)

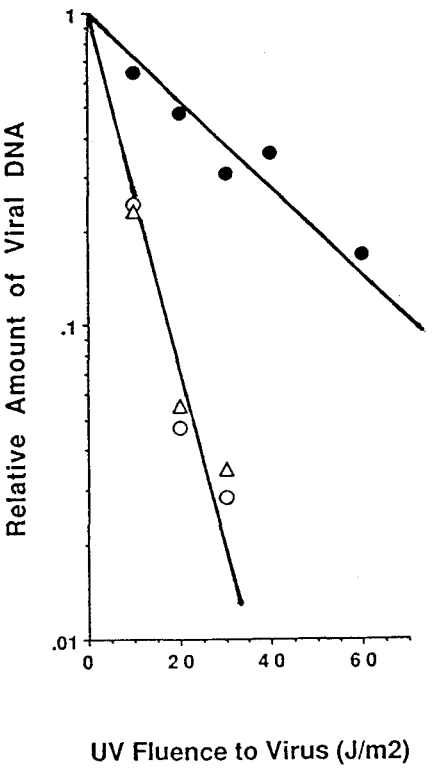
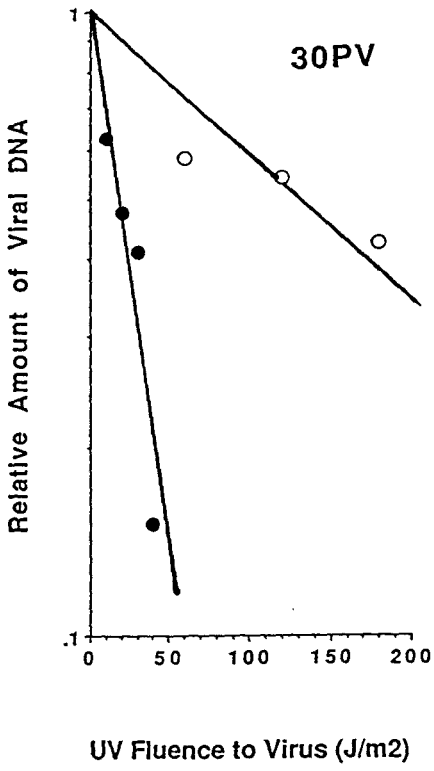
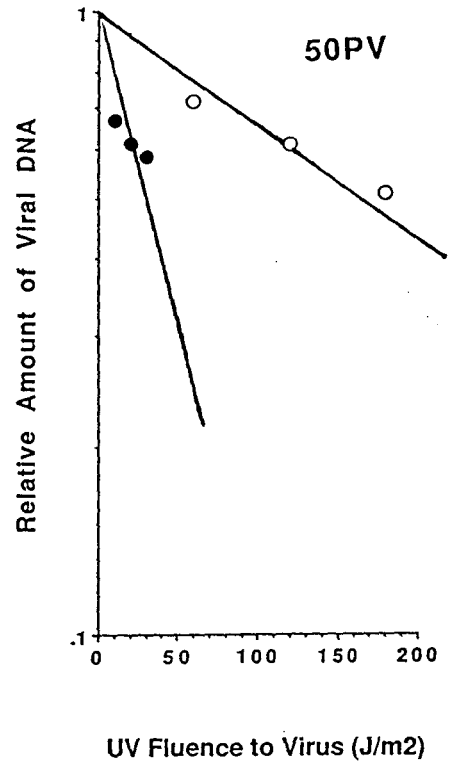

Relative Amount of Viral DNA
1
.1
.01
0
20
40
60
UV Fluence to Virus (J/m2)

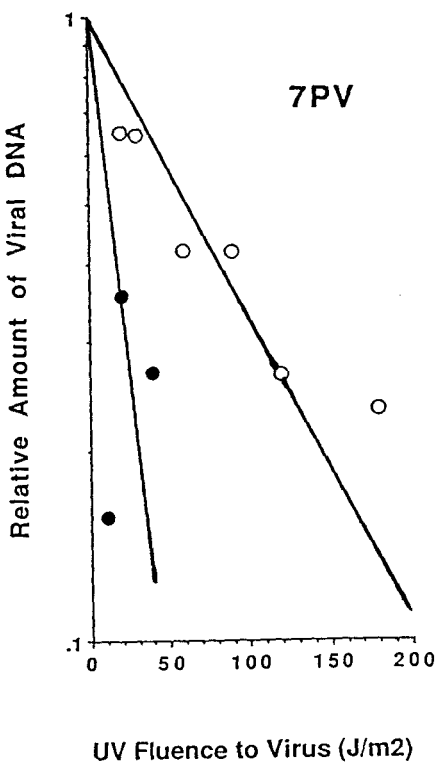
Figure 16: Survival of viral DNA synthesis in K1-derived UV-sensitive cells following infection with UV-irradiated Ad5(ERCC1)

In each experiment, which included K1 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(ERCC1) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in K1 cells and its derived DNA repair mutants. The logarithmic means of surviving fractions of viral DNA synthesis for each UV fluence from all experiments were calculated, converted to mean surviving fractions and presented in this figure. The number of experiments which were pooled for each cell line are as listed in Table 4. Ad5(LacZ) curves were included for comparison. Curves were fitted by eye.

Upper left panel:	Closed circles	- 30PV, Ad5(LacZ)
	Open circles	- 30PV, Ad5(ERCC1)
Upper right panel:	Closed circles	- 50PV, Ad5(LacZ)
	Open circles	- 50PV, Ad5(ERCC1)
Lower left panel:	Closed circles	- 7PV, Ad5(LacZ)
	Open circles	- 7PV, Ad5(ERCC1)
Lower right panel:	Closed circles	- 4PV, Ad5(LacZ)
	Open circles	- 4PV, Ad5(ERCC1)


30PV
Relative Amount of Viral DNA
1
.1
0
50
100
150
200
UV Fluence to Virus (J/m2)


50PV
Relative Amount of Viral DNA
1
.1
0
50
100
150
200
UV Fluence to Virus (J/m2)


7PV
Relative Amount of Viral DNA
1
.1
0
50
100
150
200
UV Fluence to Virus (J/m2)

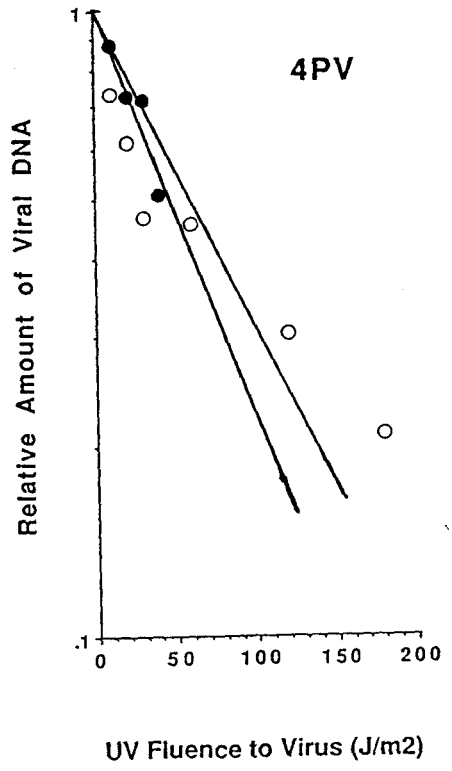

4PV
Relative Amount of Viral DNA
1
.1
0
50
100
150
200
UV Fluence to Virus (J/m2)

Figure 17: Histogram of D_0 values for viral DNA synthesis of AA8 and its derived UV-sensitive cells following UV fluence to Ad5(ERCC1)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(ERCC1) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. Linear regression analysis of pooled logarithmic surviving fractions of viral DNA synthesis from all experiments, as listed in Table 2, was used to determine D_0 values. Error bars represent standard error. Values for Ad5(LacZ) have been included for comparison.

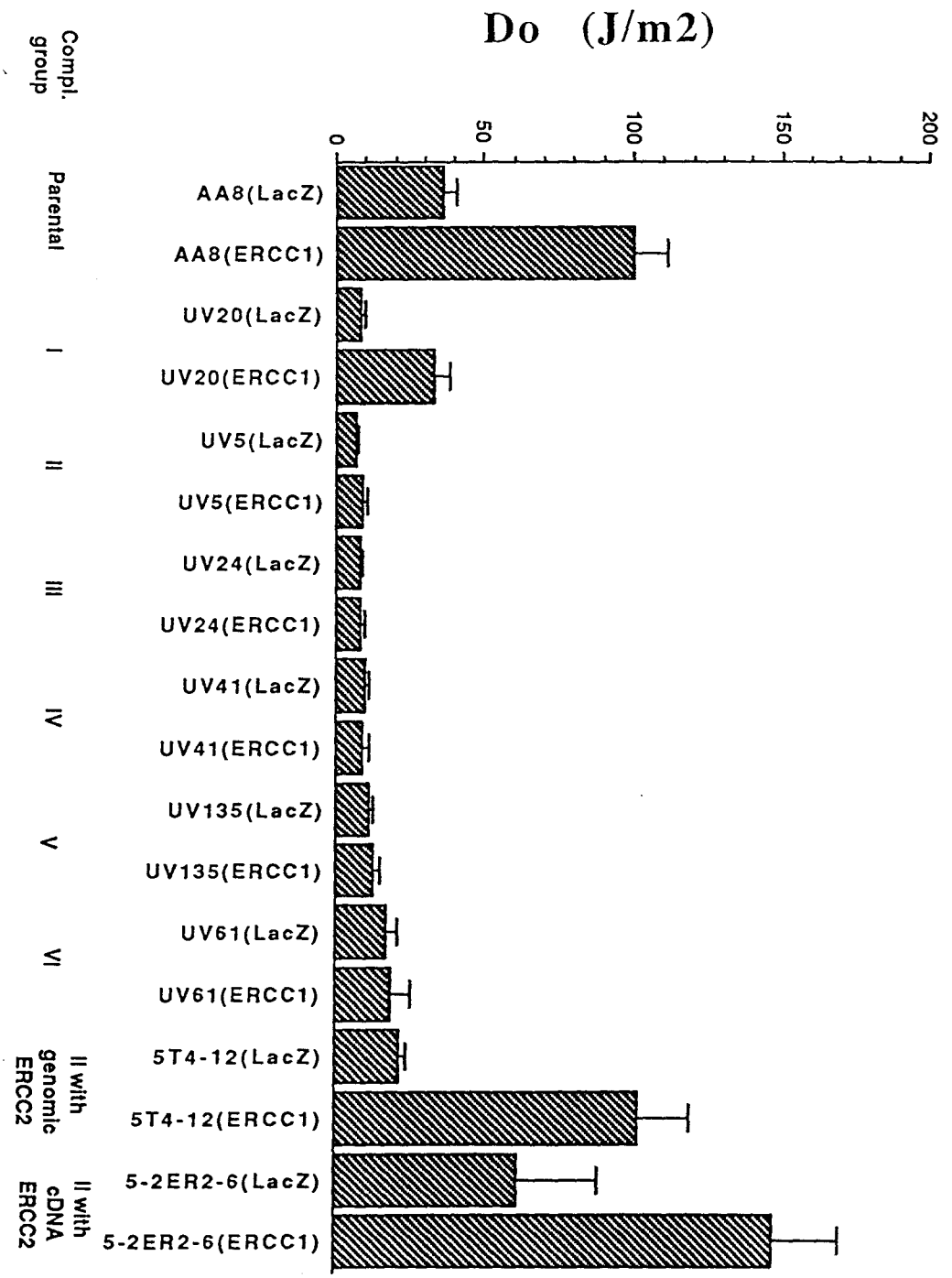

Do (J/m2)
0
50
100
150
200
Compl. group
Parental
I
II
III
IV
V
VI
II with genomic ERCC2
II with cDNA ERCC2
AA8(LacZ)
AA8(ERCC1)
UV20(LacZ)
UV20(ERCC1)
UV5(LacZ)
UV5(ERCC1)
UV24(LacZ)
UV24(ERCC1)
UV41(LacZ)
UV41(ERCC1)
UV135(LacZ)
UV135(ERCC1)
UV61(LacZ)
UV61(ERCC1)
5T4-12(LacZ)
5T4-12(ERCC1)
5-2ER2-6(LacZ)
5-2ER2-6(ERCC1)

Figure 18: Histogram of D_0 values for viral DNA synthesis of AA8 and its derived cells following UV fluence to Ad5(ERCC1)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(ERCC1) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. Linear regression analysis of pooled logarithmic surviving fractions of viral DNA synthesis from all experiments, as listed in Table 2, was used to determine D_0 values. Error bars represent standard error. Values for Ad5(LacZ) have been included for comparison.

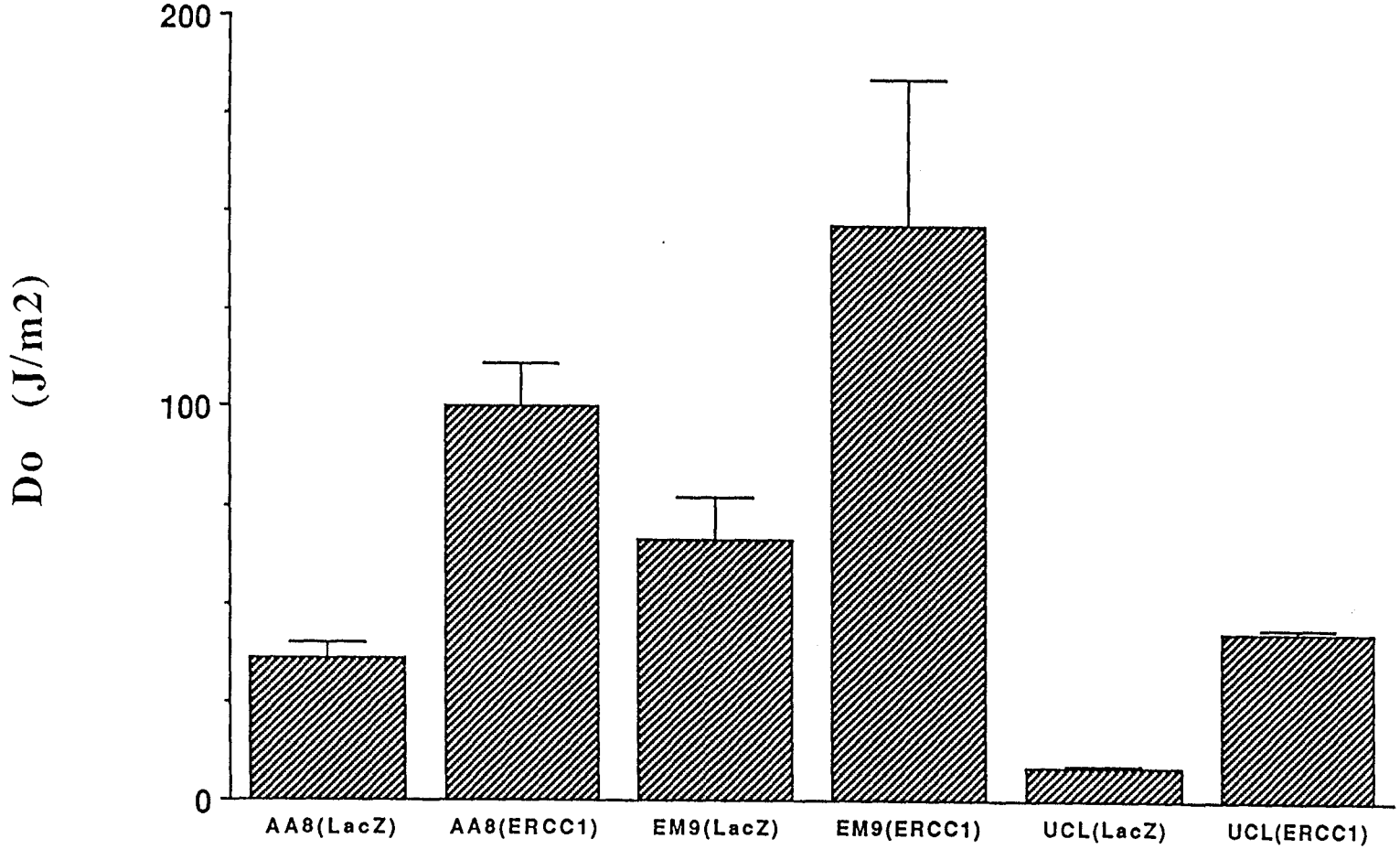
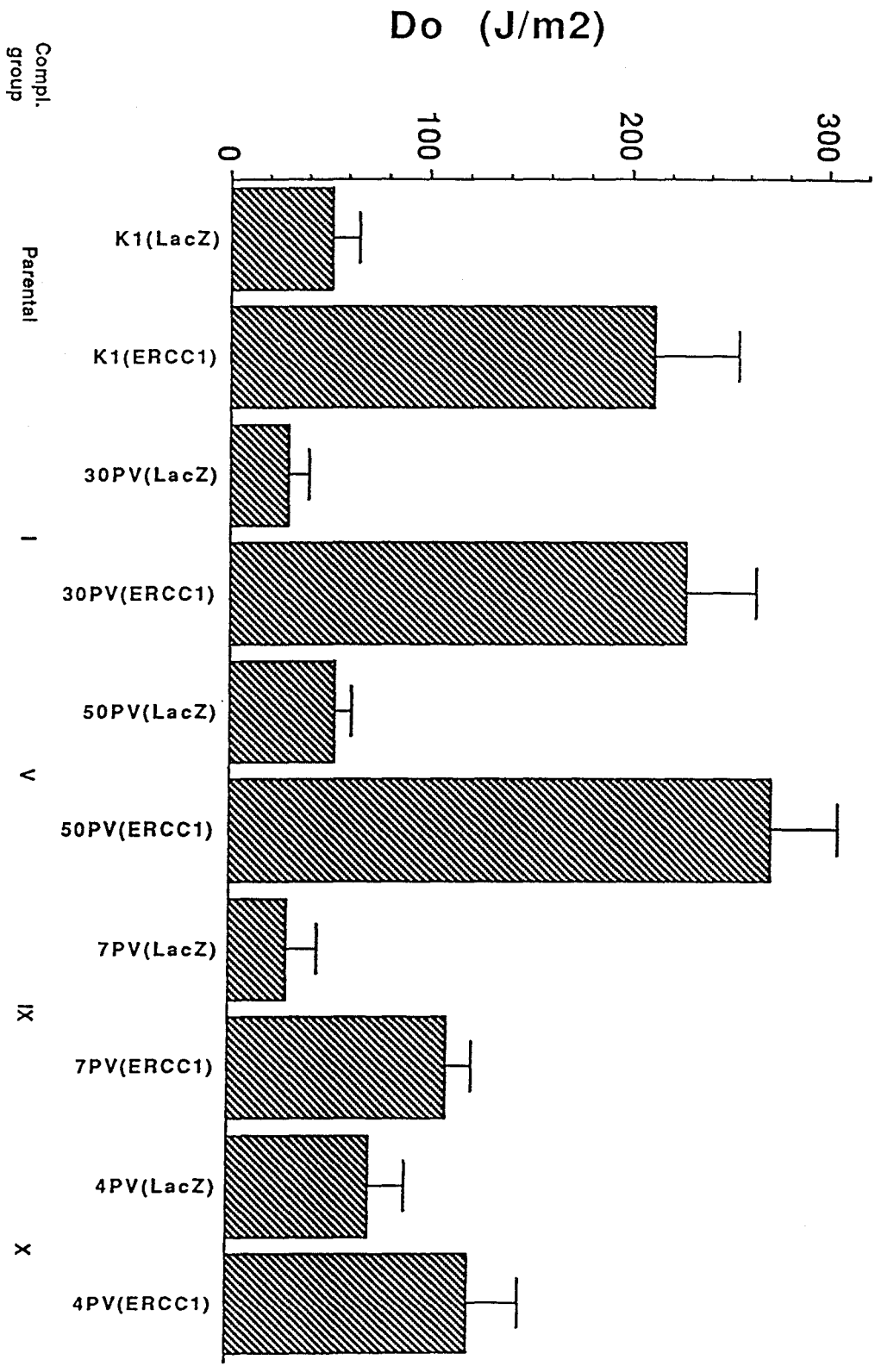

Do (J/m2)
200
100
0
AA8(LacZ)
AA8(ERCC1)
EM9(LacZ)
EM9(ERCC1)
UCL(LacZ)
UCL(ERCC1)

Figure 19: Histogram of D_0 values for viral DNA synthesis of K1 and its derived UV-sensitive cells following UV fluence to Ad5(ERCC1)

In each experiment, which included K1 and at least two mutant lines, cells were infected with UV-irradiated or non-irradiated Ad5(ERCC1) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in K1 cells and its derived DNA repair mutants. Linear regression analysis of pooled logarithmic surviving fractions of viral DNA synthesis from all experiments, as listed in Table 4, was used to determine D_0 values. Error bars represent standard error. Values for Ad5(LacZ) have been included for comparison.


Do (J/m2)
0
100
200
300
Compl. group
Parental
I
V
IX
X
K1(LacZ)
K1(ERCC1)
30PV(LacZ)
30PV(ERCC1)
50PV(LacZ)
50PV(ERCC1)
7PV(LacZ)
7PV(ERCC1)
4PV(LacZ)
4PV(ERCC1)

UV survival of Ad5(ERCC1) of EM9 and UCL were calculated and are presented in Figure 18. The UV survival of Ad5(ERCC1) is seen to be significantly increased in UV20, AA8, 5T4-12, 5-2ER2-6, EM9, and UCL with the largest effect being seen in the UCL infection (Table 5).

The UV survival of Ad5(ERCC1) DNA synthesis in K1-derived UV-sensitive cell lines was examined. The survival of viral DNA synthesis is seen to be significantly increased for K1, 30PV, 50PV, and 7PV but not 4PV following Ad5(ERCC1) infection (Figure 16; Figure 19). The ratio of D_0 - Ad5(ERCC1) to D_0 - Ad5(LacZ) demonstrate significant increases for K1, 30PV, 50PV, and 7PV although no one cell line is enhanced more than the others (Table 6).

A.4. UV Survival of Ad5(LacZ) in Human Cells

XP fibroblasts are known to be deficient in the incision step of excision repair following UV-irradiation. A deficiency in the repair of UV-irradiated Ad DNA has been detected using HCR of Vag as an assay (Rainbow 1989a). The D_{37} of Vag production following infection with UV-irradiated Ad was reduced to 6% that of normal fibroblasts.

Several human tumour cell lines have been shown to have reduced rates of incision following UV-irradiation (Squires *et al.* 1982). These repair deficiencies have been detected using HCR of Ad Vag production (Rainbow 1989c). Cervical carcinoma derived HeLa cells and colon carcinoma derived HT29 cells exhibited HCR of Vag production of 30% and 26%, respectively, compared to that of normal fibroblasts. In this light it was considered of interest

to determine if HCR of Ad DNA synthesis could detect these deficiencies in human cells.

Human fibroblasts were infected with UV-irradiated or non-irradiated Ad5(LacZ) at a MOI of 200 PFU/cell while transformed human cells were similarly infected at a MOI of 40 PFU/cell. Previous work in our laboratory has determined that synthesis of non-irradiated Ad DNA in human HeLa cells reaches its maximum by 36 to 40 hours p.i. (Rainbow and Castillo 1992). Based on these observations, viral DNA was extracted from human cells at 4 hours p.i. and 36 hours p.i. and quantified by slot-blotting, hybridization to a radioactive Ad DNA probe and scintillation counting. The net amount of viral DNA synthesized by 36 hours p.i. was determined by subtracting the amount of viral DNA quantified at 4 hours p.i. from that quantified at 36 hours p.i.

The pooled data from two experiments demonstrate a decrease in HCR of UV-irradiated Ad5(LacZ) DNA synthesis in XP1BR group D fibroblasts compared to the normal GM969 fibroblasts (Figure 20). Linear regression analysis of the pooled logarithmic surviving fractions for both experiments were used to calculate D_0 values of viral DNA synthesis for both cell types. XP1BR group D fibroblasts exhibit a HCR of D_0 of UV-irradiated Ad DNA synthesis of 7.3 $\pm$ 2.5% when compared to GM969 fibroblasts (Table 3). This value is in agreement with the reduction in HCR of Vag production in another XP group B fibroblast strain reported by Rainbow (1989a).

The logarithmic surviving fractions of viral DNA synthesis following infection of human transformed 293, HeLa, and HT29 cells with Ad5(LacZ) were determined. Pooled results from a number of experiments, as indicated in Table 3, are presented on survival curves in Figure 21. This graph indicates a

reduction in UV survival of Ad5(LacZ) in both HeLa and HT29, compared to 293 cells. The %HCR values of D_0 for UV survival of Ad5(LacZ) DNA synthesis for HeLa and HT29 were calculated to be 53.1 + 7.7% and 17.3 + 1.2%, respectively (Table 3). The deficiency reported for HeLa is not as great as the %HCR value of 30% reported by Rainbow (1989c) for Vag production but the deficiency reported here for HT29 is larger than the 26% HCR value detected by the Vag assay.

A.5. UV Survival of Ad5(denV) in Human Cells

There have been no reports of correction of reduced rates of incision in human tumour cells by the prokaryotic *denV* gene. Since increased UV survival of Ad5(denV) has been demonstrated in XP fibroblasts from groups A, C, and E (Colicos *et al.* 1991), it was of interest to examine the ability of Ad5(denV) to complement the repair deficiencies detected in human XP group D fibroblasts and transformed HeLa and HT29 cells.

Human fibroblasts were infected with UV-irradiated or non-irradiated Ad5(denV) at a MOI of 200 PFU/cell while transformed human cells were similarly infected at a MOI of 40 PFU/cell. Viral DNA was extracted from human cells at 4 hours p.i. and 36 hours p.i. and quantified by slot-blotting, hybridization to a radioactive Ad DNA probe and scintillation counting. The net amount of viral DNA synthesized by 36 hours p.i. was determined by subtracting the amount of viral DNA quantified at 4 hours p.i. from that quantified at 36 hours p.i.

Figure 20: Survival of viral DNA synthesis in GM969 (normal) fibroblasts and GM3615 (XP1BR group D) fibroblasts following infection with UV-irradiated Ad5(LacZ)

In each of two experiments, GM969 fibroblasts and XP1BR group D fibroblasts were infected with UV-irradiated or non-irradiated Ad5(LacZ) at a MOI of 200 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 36 hours p.i. The surviving fractions calculated in both experiments are presented in this figure. Curves were fitted by eye.

Closed circles - GM969, Ad5(LacZ)

Closed squares - XP1BR, Ad5(LacZ)

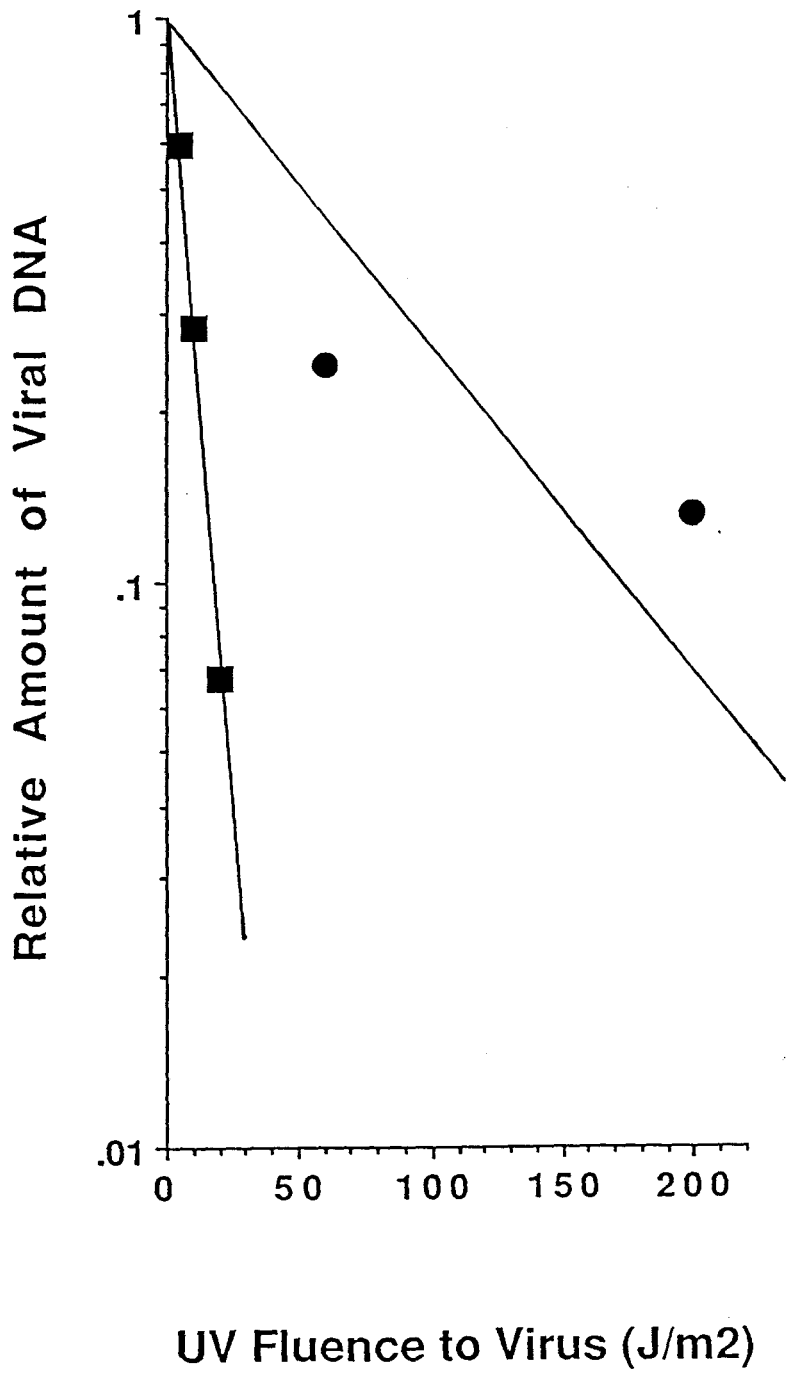
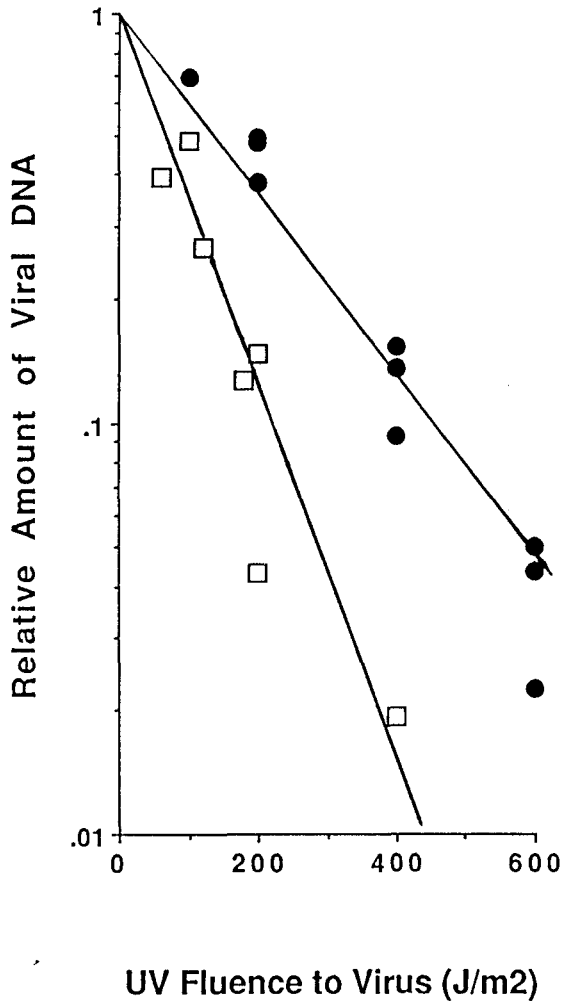

1
.1
.01
0
50
100
150
200
Relative Amount of Viral DNA
UV Fluence to Virus (J/m2)

Figure 21: Survival of viral DNA synthesis in 293, HeLa, and HT29 cells following infection with UV-irradiated Ad5(LacZ)

In individual experiments, 293 cells and HeLa and/or HT29 cells were infected with UV-irradiated or non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 36 hours p.i. in these cells. The surviving fractions of viral DNA synthesis for each UV fluence from a number of experiments, as indicated in Table 7, are presented in this figure. Curves were fitted by eye.

Left panel:	Closed circles	- 293, Ad5(LacZ)
	Open squares	- HeLa, Ad5(LacZ)
Right panel:	Closed circles	-293, Ad5(LacZ)
	Open squares	- HT29, Ad5(LacZ)


Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
UV Fluence to Virus (J/m2)

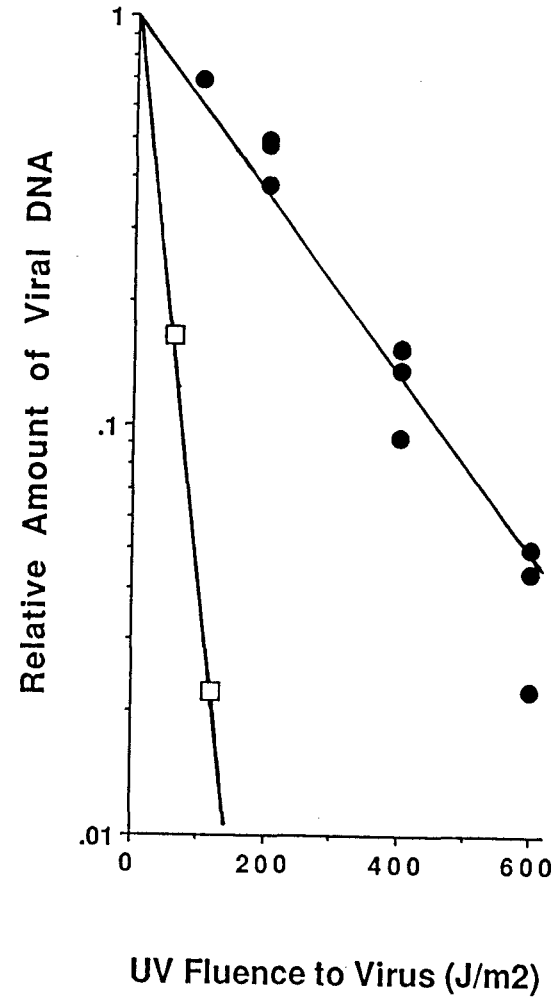
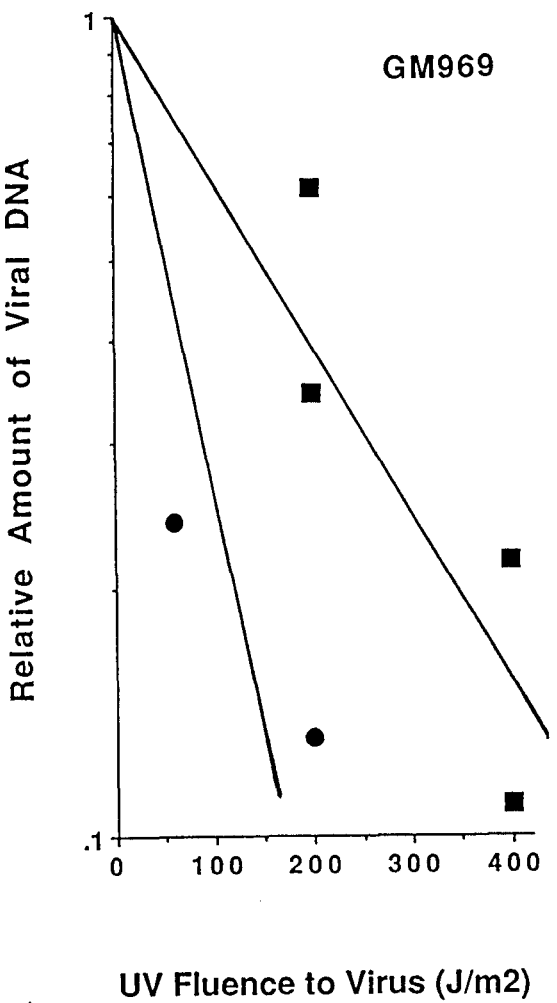

Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
UV Fluence to Virus (J/m2)

Figure 22: Survival of viral DNA synthesis in GM969 (normal) fibroblasts and GM3615 (XP1BR group D) fibroblasts following infection with UV-irradiated Ad5(denV)

In individual experiments which included GM969 and XP1BR group D fibroblasts, cells were infected with UV-irradiated or non-irradiated Ad5(denV) at a MOI of 200 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 36 hours p.i. The surviving fractions of viral DNA synthesis for each UV fluence from two experiments are presented in this figure. Ad5(LacZ) curves were included for comparison. Curves were fitted by eye.

Left panel:	Closed circles	- GM969, Ad5(LacZ)
	Closed squares	- GM969, Ad5(denV)
Right panel:	Closed circles	- XP1BR, Ad5(LacZ)
	Closed triangles	- XP1BR, Ad5(denV)


GM969
Relative Amount of Viral DNA
1
.1
0
100
200
300
400
UV Fluence to Virus (J/m2)

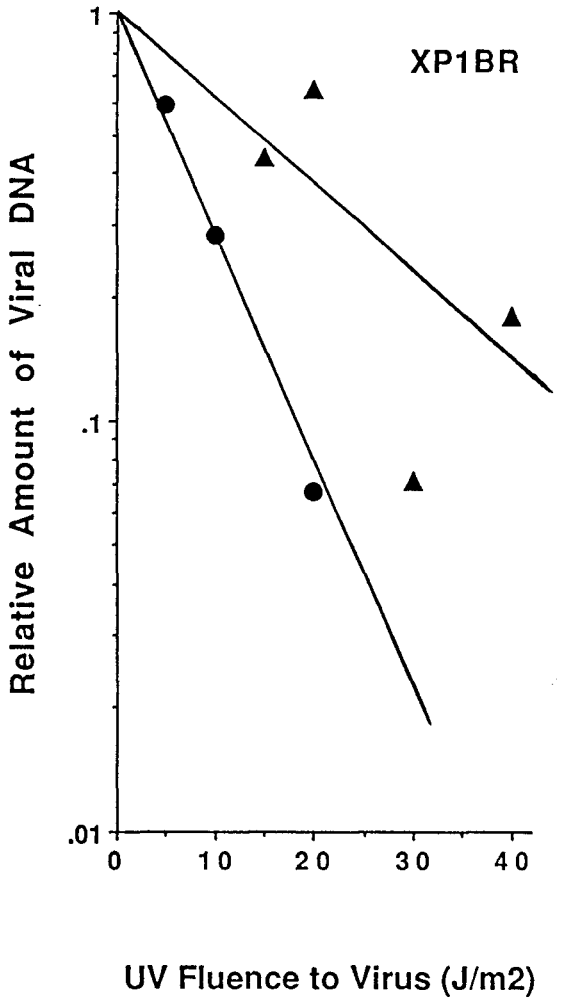
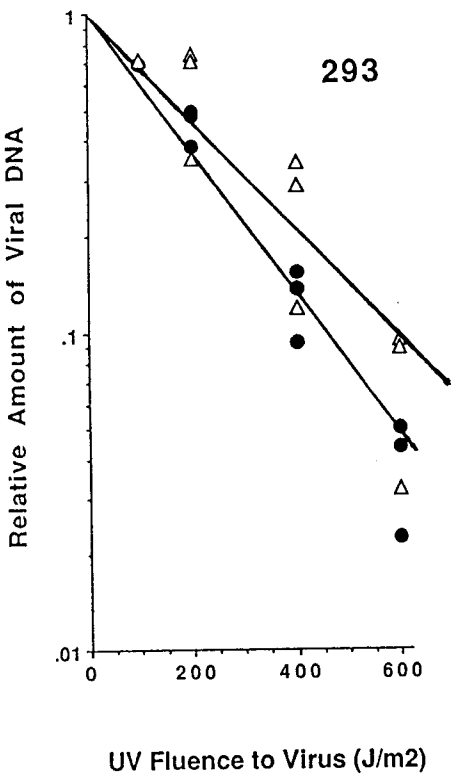
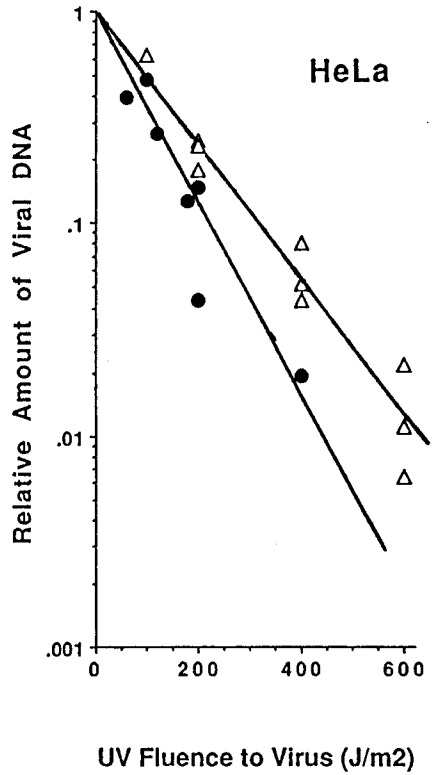

XP1BR
Relative Amount of Viral DNA
1
.1
.01
0
10
20
30
40
UV Fluence to Virus (J/m2)

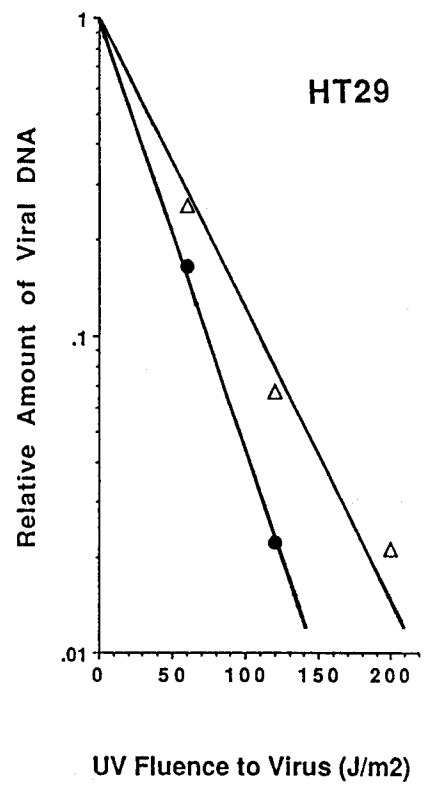
Figure 23: Survival of viral DNA synthesis in 293, HeLa, and HT29 cells following infection with UV-irradiated Ad5(denV)

In individual experiments which included 293 cells and HeLa and/or HT29 cells infection with UV-irradiated or non-irradiated Ad5(denV) was done at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 36 hours p.i. The logarithmic means of surviving fractions of viral DNA synthesis for each UV fluence from a number of experiments, as indicated in Table 7, are presented in this figure. Ad5(LacZ) curves were included for comparison. Curves were fitted by eye.

Left panel:	Closed circles	- 293, Ad5(LacZ)
	Open triangles	- 293, Ad5(denV)
Middle panel:	Closed circles	- HeLa, Ad5(LacZ)
	Open triangles	- HeLa, Ad5(denV)
Right panel:	Closed circles	- HT29, Ad5(LacZ)
	Open triangles	- HT29, Ad5(denV)


293
Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
UV Fluence to Virus (J/m2)


HeLa
Relative Amount of Viral DNA
1
.1
.01
.001
0
200
400
600
UV Fluence to Virus (J/m2)


HT29
Relative Amount of Viral DNA
1
.1
.01
0
50
100
150
200
UV Fluence to Virus (J/m2)

Increased UV survival is evident for both GM969 and XP1BR group D fibroblasts following infection with Ad5(denV) (Figure 22). The data presented in Figure 22 is not inconsistent with survival curves possessing a shoulder at low UV fluences. However, the data was also consistent with simple exponential analysis and linear regression analysis was used to determine the D_0 values of UV survival of viral DNA synthesis (Table 7). The ratio of D_0 - Ad5(denV) to D_0 - Ad5(LacZ) for these fibroblasts reveal that UV survival of both is increased significantly (Table F).

The survival curves presented in Figure 23 demonstrate small increases in UV survival of Ad5(denV) DNA synthesis for 293, HeLa, and HT29 cell lines compared to Ad5(LacZ). The small significant increases in UV survival of Ad5(denV) compared to Ad5(LacZ) of these transformed cells range from 1.24 ± 0.20 to 1.63 ± 0.13 for 293 and HT29, respectively. These increases are approximately 60% of the increases found for the fibroblasts (Table 7).

A.6. UV Survival of Ad5(ERCC1) Human Cells

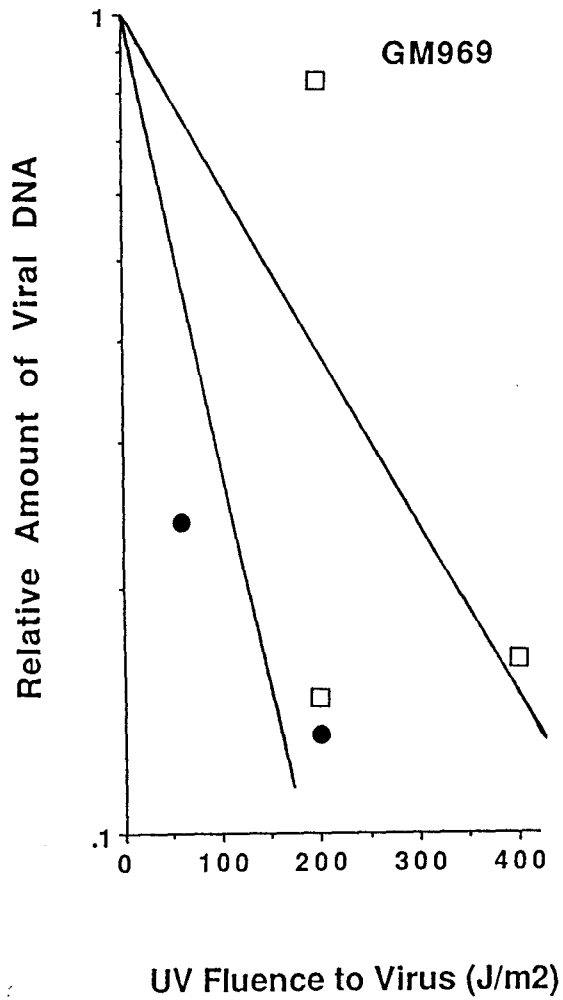
There have been no published reports of the effects of adding exogenous *ERCC1* to human cells and as such it was considered of interest to examine the UV survival of Ad5(ERCC1) DNA synthesis in human fibroblasts and human transformed cell lines.

Human fibroblasts were infected with UV-irradiated or non-irradiated Ad5(ERCC1) at a MOI of 200 PFU/cell while transformed human cells were similarly infected at a MOI of 40 PFU/cell. Viral DNA was extracted from human cells at 4 hours p.i. and 36 hours p.i. and quantified by slot-blotting,

Figure 24: Survival of viral DNA synthesis in GM969 (normal) cells and GM3615 (XP1BR group D) following infection with UV-irradiated Ad5(ERCC1)

In individual experiments which included GM969 and XP1BR group D fibroblasts, cells were infected with UV-irradiated or non-irradiated Ad5(ERCC1) at a MOI of 200 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 36 hours p.i. The surviving fractions of viral DNA synthesis for each UV fluence from two experiments are presented in this figure. Ad5(LacZ) curves were included for comparison. Curves were fitted by eye.

Left panel:	Closed circles	- GM969, Ad5(LacZ)
	Open squares	- GM969, Ad5(ERCC1)
Right panel:	Closed circles	- XP1BR, Ad5(LacZ)
	Open squares	- XP1BR, Ad5(ERCC1)


GM969
Relative Amount of Viral DNA
1
.1
0
100
200
300
400
UV Fluence to Virus (J/m2)

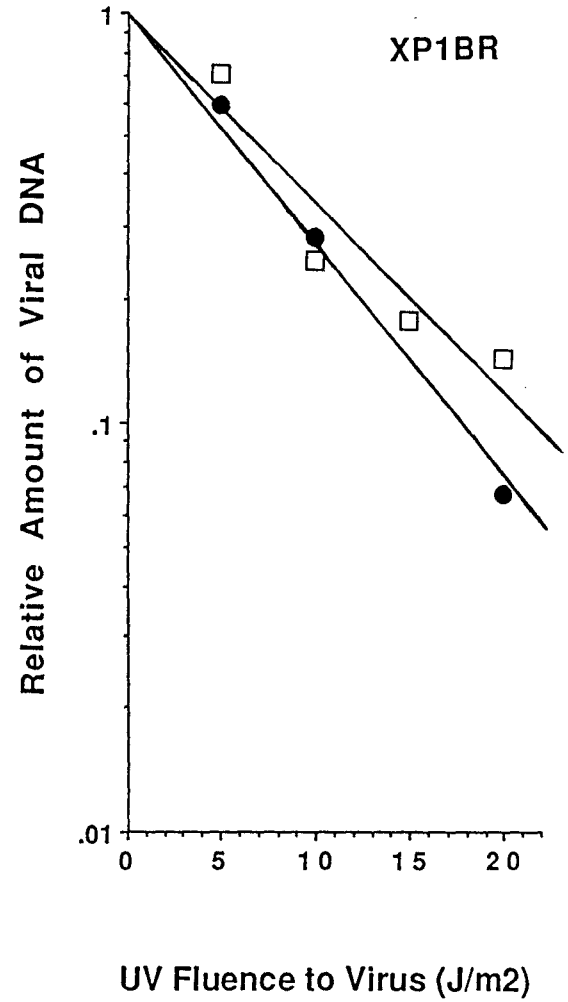
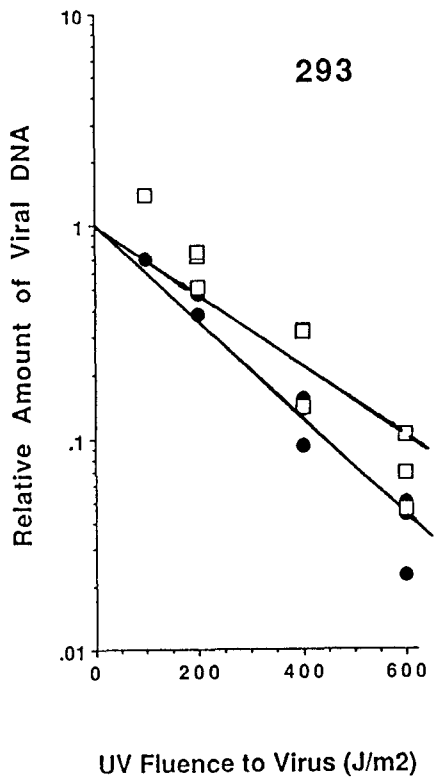
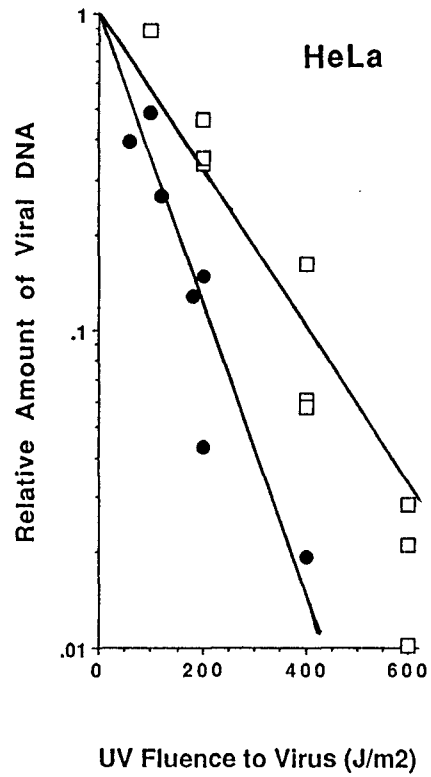

XP1BR
Relative Amount of Viral DNA
1
.1
.01
0
5
10
15
20
UV Fluence to Virus (J/m2)

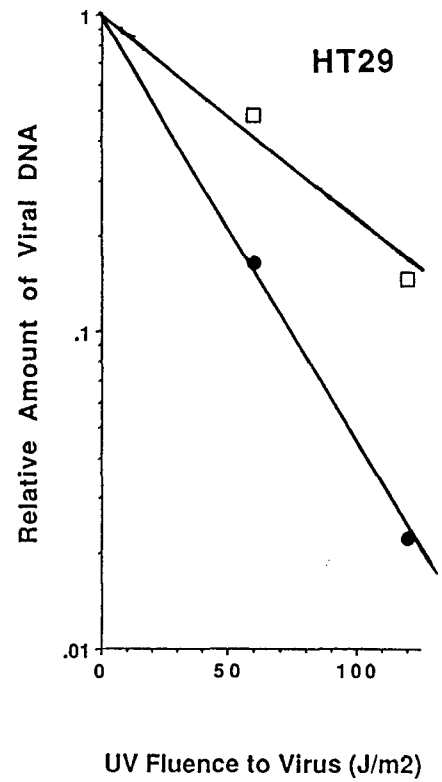
Figure 25: Survival of viral DNA synthesis in 293, HeLa, and HT29 cells following infection with UV-irradiated Ad5(ERCC1)

In individual experiments which included 293 cells and HeLa and/or HT29 cells, infection with UV-irradiated or non-irradiated Ad5(ERCC1) was done at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 36 hours p.i. The surviving fractions of viral DNA synthesis for each UV fluence from a number of experiments, as indicated in Table 7, are presented in this figure. Ad5(LacZ) curves were included for comparison. Curves were fitted by eye.

Left panel:	Closed circles	- 293, Ad5(LacZ)
	Open squares	- 293, Ad5(ERCC1)
Middle panel:	Closed circles	- HeLa, Ad5(LacZ)
	Open squares	- HeLa, Ad5(ERCC1)
Right panel:	Closed circles	- HT29, Ad5(LacZ)
	Open squares	- HT29, Ad5(ERCC1)


293
Relative Amount of Viral DNA
10
1
.1
.01
0
200
400
600
UV Fluence to Virus (J/m2)


HeLa
Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
UV Fluence to Virus (J/m2)


HT29
Relative Amount of Viral DNA
1
.1
.01
0
50
100
UV Fluence to Virus (J/m2)

hybridization to a radioactive Ad DNA probe and scintillation counting. The net amount of viral DNA synthesized by 36 hours p.i. was determined by subtracting the amount of viral DNA quantified at 4 hours p.i. from that quantified at 36 hours p.i.

The surviving fractions of Ad5(ERCC1) DNA synthesis from two experiments were pooled and are displayed in Figure 24. These graphs demonstrate that UV survival of Ad5(ERCC1) is enhanced to a much greater extent compared to that of Ad5(LacZ) in the normal GM969 fibroblasts than in XP1BR group D fibroblasts. Analysis of the logarithmic surviving fractions by linear regression was used to determine that the D_0 for Ad5(ERCC1) DNA synthesis in GM969 is 215.00 $\pm$ 98.49 J/m^2 and 9.44 $\pm$ 1.08 J/m^2 in XP1BR group D fibroblasts. This represents an enhancement of UV survival of Ad5(ERCC1) DNA synthesis in GM969 and XP1BR fibroblasts of 2.15 $\pm$ 1.64 and 1.28 $\pm$ 0.19, respectively (Table 8).

Figure 25 demonstrates the UV survival of Ad5(ERCC1) in transformed human cells compared to that of Ad5(LacZ). A survival curve with a shoulder at low UV fluences appears to fit the data, however, it is also consistent with a simple exponential decrease as a function of UV fluence. Linear regression analysis of the pooled logarithmic surviving fractions of viral DNA synthesis was used to calculate the D_0 values of Ad5(ERCC1) DNA synthesis (Table 7). A calculation of the ratio of the D_0 for Ad5(ERCC1) to that of Ad5(LacZ) for UV-irradiated virus for the three cell lines reveals a value of 2.06 $\pm$ 0.22 for HT29, 1.54 $\pm$ 0.25 for HeLa, and 1.22 $\pm$ 0.19 for 293 (Table 8).

The enhancement of UV survival of Ad5(ERCC1) DNA synthesis in the apparently DNA repair-proficient 293 cells (Rainbow 1989) is found to be

lessthan in the normal GM969 fibroblasts. The enhancement of viral DNA synthesis in XP1BR group D fibroblasts due to ERCC1 is the smallest of the human cells.

B. Survival of Viral DNA Synthesis for γ-Irradiated Adenovirus In Mammalian Cells

The spectrum of DNA damage induced by γ-rays is known to be different from that induced by UV irradiation. This difference is pronounced to a greater extent when the γ-irradiation is carried out at 0^0C compared to -75^0C (Bennett and Rainbow 1989). These authors report that HCR of Ad Vag production in an XP fibroblast strain was reduced to 57% of normal when the virus was irradiated at -75^0C. However, irradiation of the virus at 0^0C resulted in a reduction in HCR of Vag production in this same XP strain to only 88% of normal.

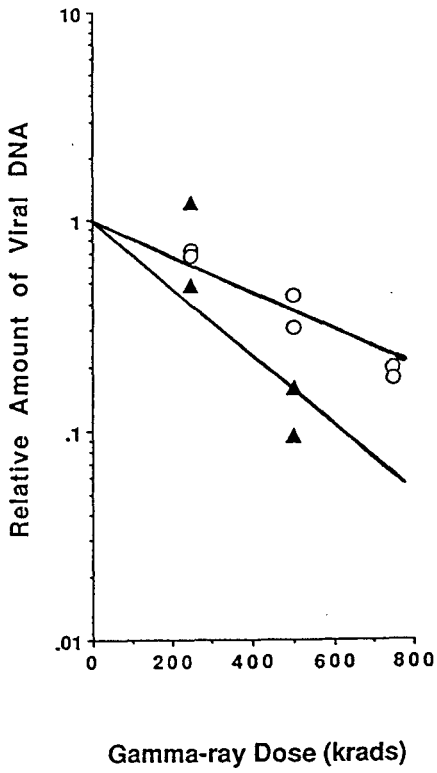
Rubin and Whitmore (1985) report that UV20 cells treated with γ-rays under aerobic conditions do not differ from parental cells in terms of their CFA. However, UV20 which are γ-irradiated under hypoxic conditions were found to exhibit an increased sensitivity for CFA compared to parental cells. Irradiation of UV5 cells with γ-rays under aerobic conditions was not found to decrease the survival of these cells compared to the parental AA8 although the D_0 for survival of EM9-1 cells was reduced to 63% that of AA8 (Thompson *et al.* 1980).

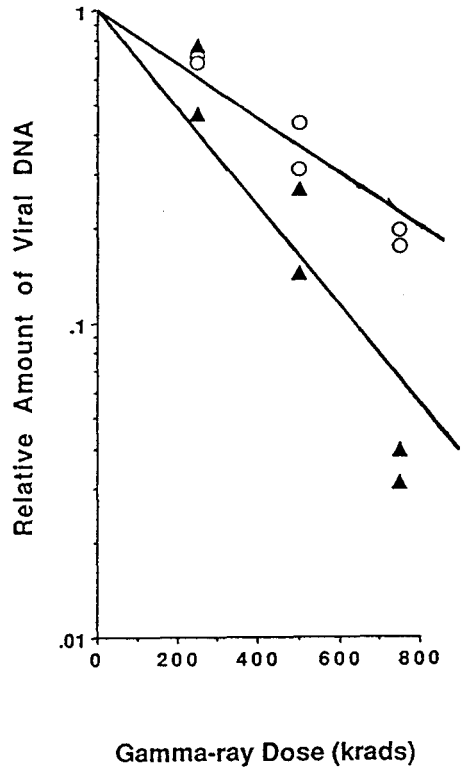
It was considered of interest to determine if the spectrum of damage induced by γ-irradiation could be detected in CHO cells using HCR of Ad DNA

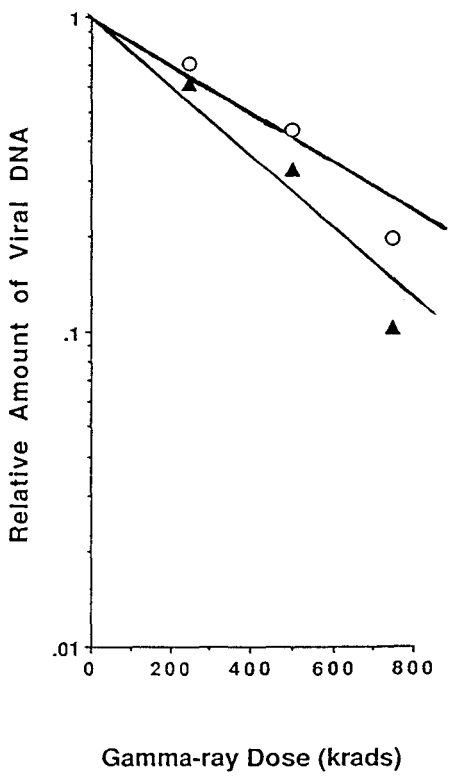
Figure 26: Survival of viral DNA synthesis in CHO cells following infection with γ-irradiated Ad5(LacZ)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with γ-irradiated or non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. The surviving fractions of viral DNA synthesis for each γ-ray dose from a number of experiments, as indicated in Table 9, are presented in this figure. Curves were fitted by eye.

Upper left panel:	Open circles	- AA8, Ad5(LacZ)
	Closed triangles	- UV20, Ad5(LacZ)
Upper right panel:	Open circles	- AA8, Ad5(LacZ)
	Closed triangles	- UV41, Ad5(LacZ)
Lower left panel:	Open circles	- AA8, Ad5(LacZ)
	Closed triangles	- EM9, Ad5(LacZ)
Lower right panel:	Open circles	- AA8, Ad5(LacZ)
	Closed triangles	- UV5, Ad5(LacZ)


Relative Amount of Viral DNA
10
1
.1
.01
0
200
400
600
800
Gamma-ray Dose (krads)


Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
800
Gamma-ray Dose (krads)


Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
800
Gamma-ray Dose (krads)

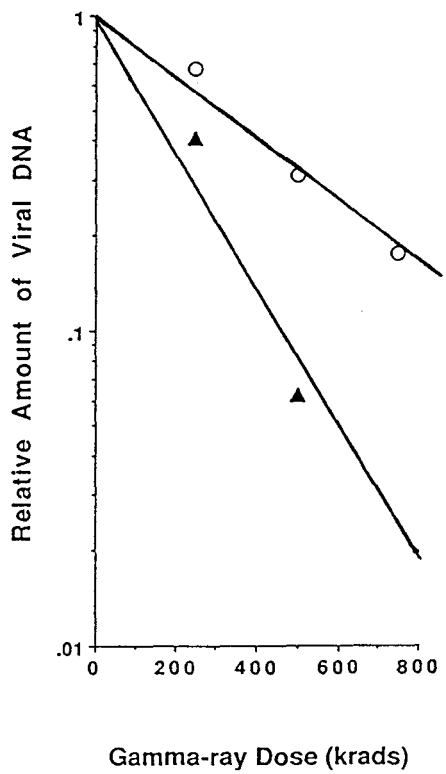
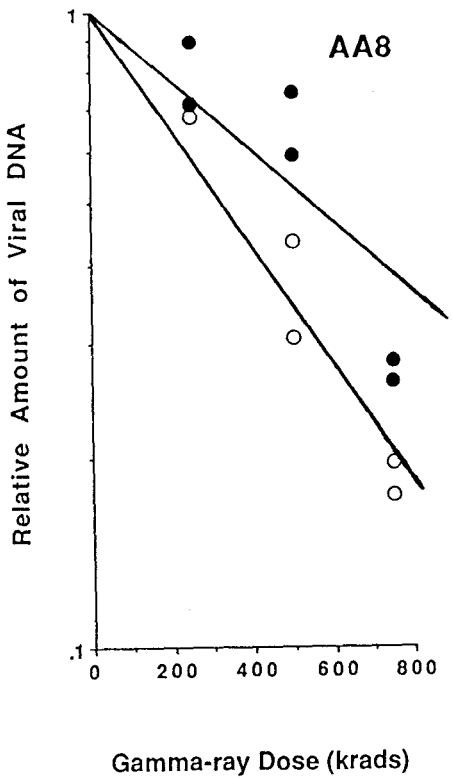
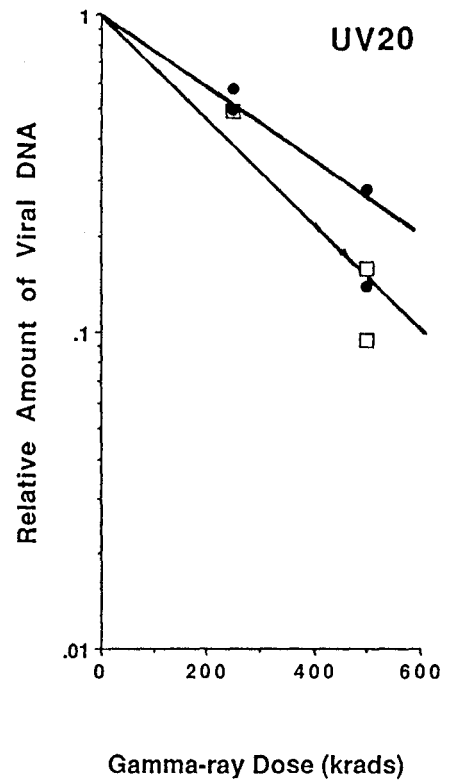

Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
800
Gamma-ray Dose (krads)

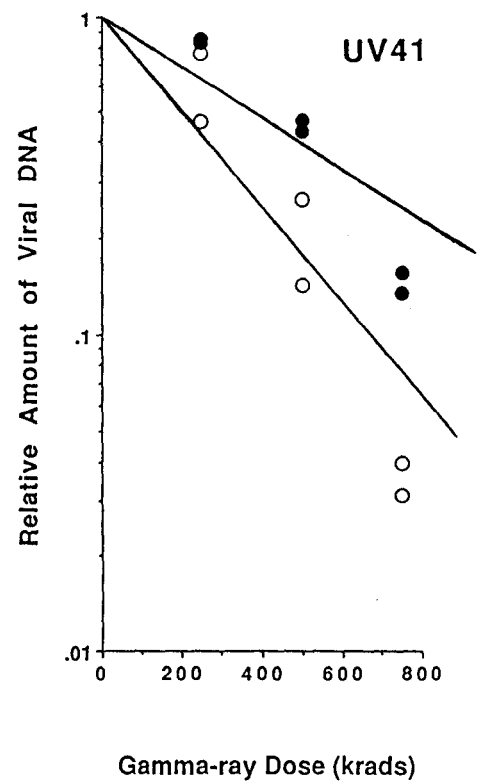
Figure 27: Survival of viral DNA synthesis in CHO cells following infection with γ-irradiated Ad5(denV)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with γ-irradiated or non-irradiated Ad5(denV) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. The surviving fractions of viral DNA synthesis for each γ-ray dose from a number of experiments, as indicated in Table 9, are presented in this figure. Survival curves for Ad5(LacZ) were included for comparison. Curves were fitted by eye.

Left panel:	Open circles	- AA8, Ad5(LacZ)
	Closed circles	- AA8, Ad5(denV)
Middle panel:	Open circles	- UV20, Ad5(LacZ)
	Closed circles	- UV20, Ad5(denV)
Right panel:	Open circles	- UV41, Ad5(LacZ)
	Closed circles	- UV41, Ad5(denV)


AA8
Relative Amount of Viral DNA
1
.1
0
200
400
600
800
Gamma-ray Dose (krads)


UV20
Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
Gamma-ray Dose (krads)


UV41
Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
800
Gamma-ray Dose (krads)

synthesis. The ability of *ERCC1* and *denV* to correct any deficiencies detected was also thought to be of interest.

B.1. γ-ray Survival of Ad5(LacZ) in Normal and DNA Repair Deficient CHO Cells

In each experiment, which included a parental cell line and at least two mutant cell lines, cells were infected with either γ-irradiated or non-irradiated Ad5(LacZ) and at 4 hours and 72 hours p.i., the viral DNA was extracted from the cells and quantified by slot-blotting, hybridization to a radioactive probe and scintillation counting. The net viral DNA synthesis was determined by subtracting the input viral DNA (4 hour p.i.) from the amount determined at 72 hours p.i. In individual experiments, the net viral DNA produced for each γ-ray dose to the virus was expressed as a fraction of that produced in cells infected with unirradiated virus.

Figure 26 demonstrates reductions in γ-ray survival of Ad5(LacZ) DNA synthesis in UV20, UV5, and UV41 which all appear to be greater than the reduction seen in EM9. Linear regression analysis of the pooled logarithmic surviving fractions of viral DNA synthesis indicate reductions in γ-ray survival of Ad(LacZ) DNA synthesis to approximately 50% that of parental for the three UV-sensitive mutants whereas the calculated D_0 of 335.10 ± 48.21 krads (Table 9) is 77% that of AA8.

B.2. γ-ray Survival of Ad5(denV) in Normal and DNA Repair Deficient CHO Cells

The DNA damage spectrum induced by γ-rays differs from that induced by UV light (Bennett and Rainbow 1989). As *denV* is specific for PDs which are one of the major photoproducts induced by UV-irradiation (Friedberg and King 1971), it was considered of interest to examine the γ-ray survival of Ad5(denV) DNA synthesis in CHO cells.

In each experiment, which included a parental cell line and at least two mutant cell lines, cells were infected with either γ-irradiated or non-irradiated Ad5(denV) and at 4 hours and 72 hours p.i., the viral DNA was extracted from the cells and quantified by slot-blotting, hybridization to a radioactive probe and scintillation counting. The net viral DNA synthesis was determined by subtracting the input viral DNA (4 hour p.i.) from the amount determined at 72 hours p.i. In individual experiments, the net viral DNA produced for each γ-ray dose to the virus was expressed as a fraction of that produced in cells infected with unirradiated virus.

The pooled surviving fractions of Ad5(denV) DNA synthesis following infection of CHO cells with γ-irradiated virus for a number of experiments, as listed in Table 10, are plotted in Figure 27 and Figure 28. The survival of Ad5(denV) DNA synthesis is seen to be significantly enhanced in AA8, UV41, UV5, and EM9 (Table 10).

B.3. γ-ray Survival of Ad5(ERCC1) in Normal and DNA Repair Deficient CHO Cells

Although *ERCC1* has been shown to be involved in the repair of UV- and MMC-induced DNA damage (Westerveld *et al.* 1984), there is littleinformation avalailable concerning the role of *ERCC1* in repair of γ-ray induced DNA damage. As such it was thought to be important to determine the role of *ERCC1* in this type of repair by examining the HCR of γ-irradiated Ad5(ERCC1) in CHO cells.

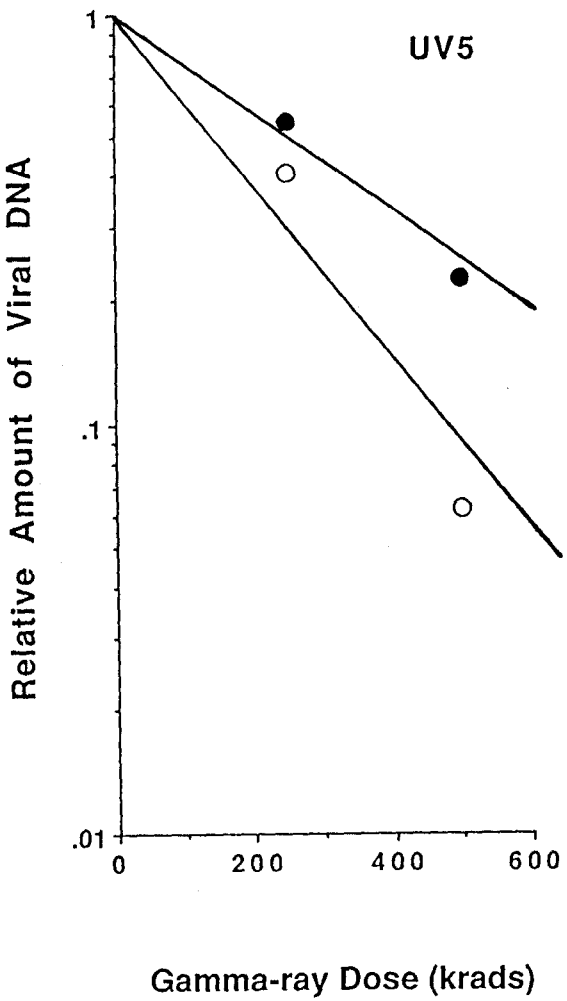
In each experiment, which included a parental cell line and at least two mutant cell lines, cells were infected with either γ-irradiated or non-irradiated Ad5(ERCC1) and at 4 hours and 72 hours p.i., the viral DNA was extracted from the cells and quantified by slot-blotting, hybridization to a radioactive probe and scintillation counting. The net viral DNA synthesis was determined by subtracting the input viral DNA (4 hour p.i.) from the amount determined at 72 hours p.i. In individual experiments, the net viral DNA produced for each γ-ray dose to the virus was expressed as a fraction of that produced in cells infected with unirradiated virus.

The γ-ray survival of Ad5(ERCC1) is seen to be increased in UV20, UV41, and EM9 but not in AA8 or UV5 (Figures 29 and 30). Linear regression analysis of pooled logarithmic surviving fractions from a number of experiments, as indicated in Table 10, was used to calculate D_0 values for γ-ray survival of Ad5(ERCC1) DNA synthesis. The ratios of D_0 - Ad5(ERCC1) to D_0 - Ad5(LacZ) are summarized in Table 10.

Figure 28: Survival of viral DNA synthesis in CHO cells following infection with γ-irradiated Ad5(denV)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with γ-irradiated or non-irradiated Ad5(denV) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. The surviving fractions of viral DNA synthesis for each γ-ray dose from a number of experiments, as indicated in Table 9, are presented in this figure. Survival curves for Ad5(LacZ) were included for comparison. Curves were fitted by eye.

Left panel:	Open circles	- UV5, Ad5(LacZ)
	Closed circles	- UV5, Ad5(denV)
Right panel:	Open circles	- EM9, Ad5(LacZ)
	Closed circles	- EM9, Ad5(denV)


UV5
Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
Gamma-ray Dose (krads)

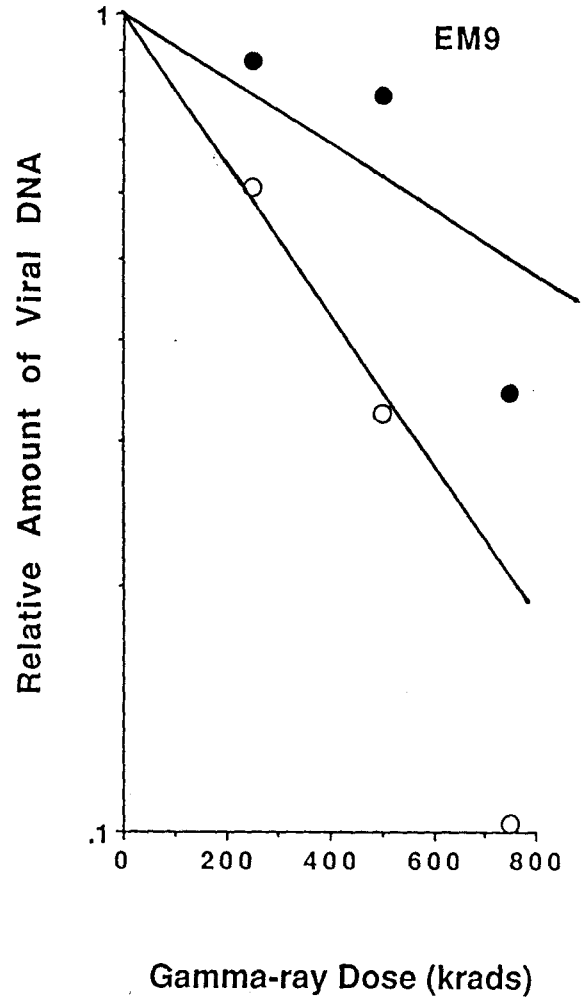

EM9
Relative Amount of Viral DNA
1
.1
0
200
400
600
800
Gamma-ray Dose (krads)

Figure 29: Survival of viral DNA synthesis in CHO cells following infection with γ-irradiated Ad5(ERCC1)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with γ-irradiated or non-irradiated Ad5(ERCC1) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. The surviving fractions of viral DNA synthesis for each γ-ray dose from a number of experiments, as indicated in Table 9, are presented in this figure. Survival curves for Ad5(LacZ) were included for comparison. Curves were fitted by eye.

Left panel:	Open circles	- AA8, Ad5(LacZ)
	Closed circles	- AA8, Ad5(ERCC1)
Middle panel:	Open circles	- UV20, Ad5(LacZ)
	Closed circles	- UV20, Ad5(ERCC1)
Right panel:	Open circles	- UV41, Ad5(LacZ)
	Closed circles	- UV41, Ad5(ERCC1)

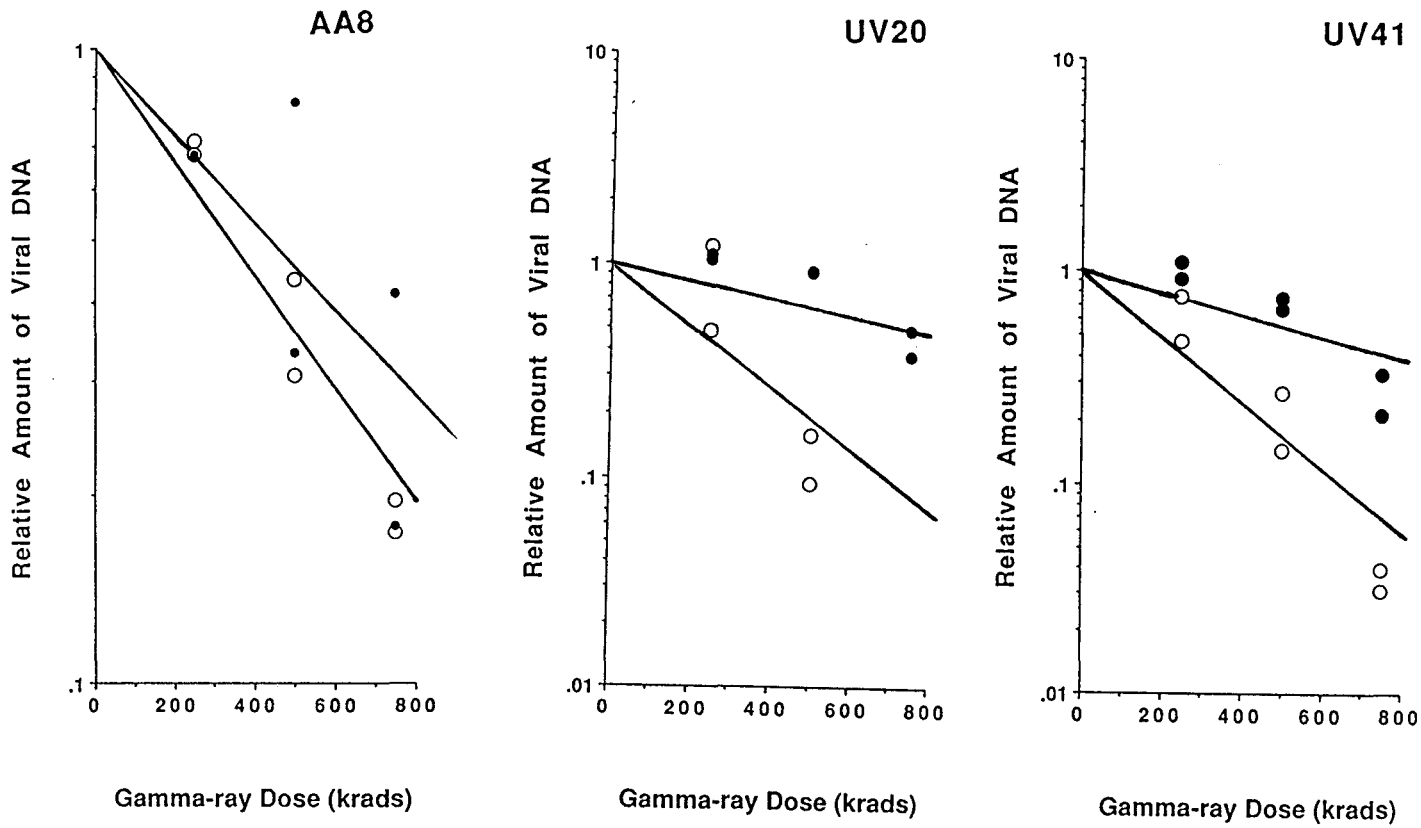
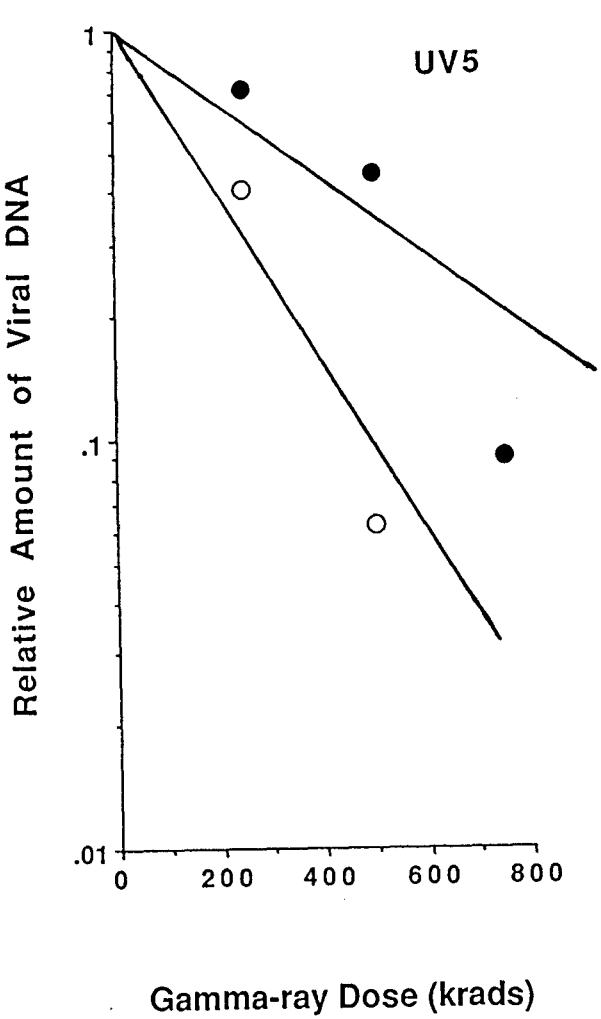
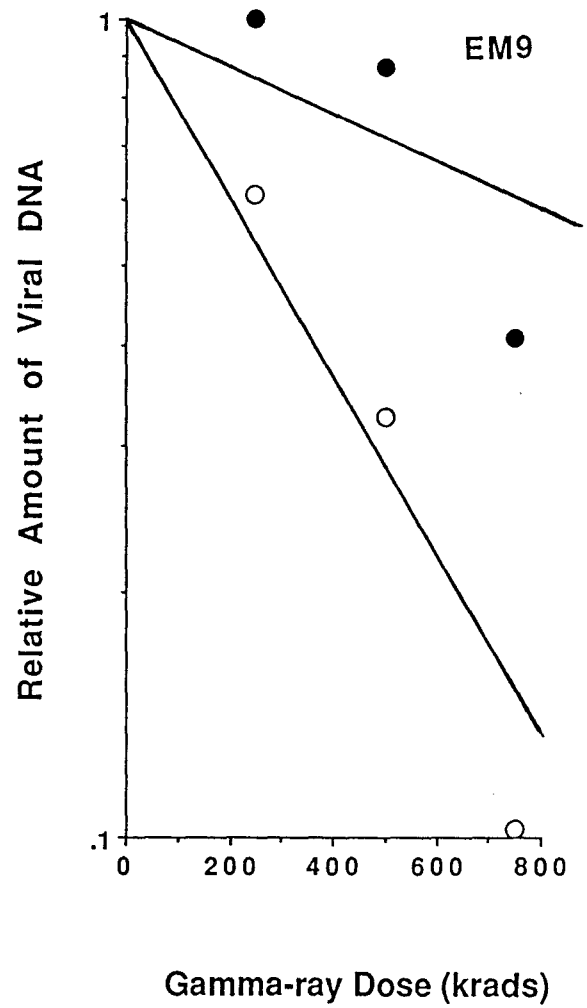

AA8
UV20
UV41
Relative Amount of Viral DNA
Gamma-ray Dose (krads)
1
.1
10
.01
0
200
400
600
800

Figure 30: Survival of viral DNA synthesis in CHO cells following infection with γ-irradiated Ad5(ERCC1)

In each experiment, which included AA8 and at least two mutant lines, cells were infected with γ-irradiated or non-irradiated Ad5(ERCC1) at a MOI of 40 PFU/cell. The surviving fraction of viral DNA synthesis was then scored at 72 hours p.i. in AA8 cells and its derived DNA repair mutants. The surviving fractions of viral DNA synthesis for each γ-ray dose from a number of experiments, as indicated in Table 9, are presented in this figure. Survival curves for Ad5(LacZ) were included for comparison. Curves were fitted by eye.

Left panel:	Open circles	- UV5, Ad5(LacZ)
	Closed circles	- UV5, Ad5(ERCC1)
Right panel:	Open circles	- EM9, Ad5(LacZ)
	Closed circles	- EM9, Ad5(ERCC1)


UV5
Relative Amount of Viral DNA
1
.1
.01
0
200
400
600
800
Gamma-ray Dose (krads)


EM9
Relative Amount of Viral DNA
1
.1
0
200
400
600
800
Gamma-ray Dose (krads)

C. Adenovirus Replication in Mammalian Cells

During the initial experiments which investigated the amounts of viral DNA produced in CHO cells, it was observed that some of the UV-sensitive CHO mutants, such as UV20, were deficient in the amount of viral DNA produced by 72 hours post-infection compared to AA8 (Figure 5). As a result, it was considered to be of interest to further characterize the viral DNA replicative abilities of the CHO cells being used in this study.

C.1. Replication of Ad5(LacZ) DNA in Normal and Repair-Deficient CHO Cells

Each experiment performed included a parental cell line and at least two mutants cell lines. Cells were infected with non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA extracted from the cells at 4 hours p.i. and 72 hours p.i. Quantification of viral DNA was done by slot-blotting, hybridization to a radioactive Ad DNA probe and scintillation counting. The net amount of viral DNA synthesized by 72 hours p.i. was determined by subtracting the amount of viral DNA at 4 hours p.i. from the quantity found at 72 hours p.i. The uptake of virus by each cell line in individual experiments was assessed by examining the relative amounts of viral DNA scored at the 4 hour p.i. time points. This method of assessment of viral uptake did not reveal any differences between the cell lines.

In individual experiments, the net amount of viral DNA synthesized in each cell line was expressed as a percentage of that synthesized in AA8. In a number of experiments, the effect of different MOI values was examined. It was

determined that a deficiency in viral DNA replication was more pronounced for a lower MOI compared to a higher MOI (data not shown).

The average replication of Ad5(LacZ) DNA for AA8 and its UV-sensitive mutants in number of experiments, as indicated in Table 3, was calculated (Table 3) and presented in Figure 31. This figure demonstrates that UV20, UV24, and UV61 are UV-sensitive mutants derived from AA8 which exhibit a significant reduction in replication of Ad5(LacZ) DNA. The 5T4-12 cell line was also found to have a significant reduction in viral DNA replication.

Replication of Ad5(LacZ) DNA was significantly reduced in UCL compared to that in AA8 (Figure 32, Table 11). EM9 was found to have similar viral DNA replication capabilities compared to AA8 (Figure 32, Table 11).

A significant reduction in replication of Ad5(LacZ) DNA synthesis was found for 7PV compared to parental K1 while the other mutants were within wild-type levels (Figure 33). The values obtained for K1 and its mutants are listed in Table 12.

C.2. Replication of Ad5(denV) DNA in Normal and Repair-Deficient CHO Cells

The bacteriophage T4 *denV* gene is known to encode a protein which incises DNA at sites of PDs. Although there has been no evidence to suggest a role for *denV* in DNA replication, observations during initial experiments of this study indicated that replication of Ad5(denV) DNA was increased compared to that of Ad5(LacZ) in CHO cells. It was therefore considered worthwhile to

Figure 31: Histogram of relative viral DNA synthesis of AA8 and its derived UV-sensitive cells following infection with unirradiated Ad5(LacZ)

In individual experiments, which included AA8 and at least two mutant lines, cells were infected with unirradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 72 hours post-infection for each cell line, relative to AA8. The mean relative viral DNA synthesis of each cell line for a number of experiments, as indicated in Table11, was calculated and is presented in this figure. Error bars are standard error.

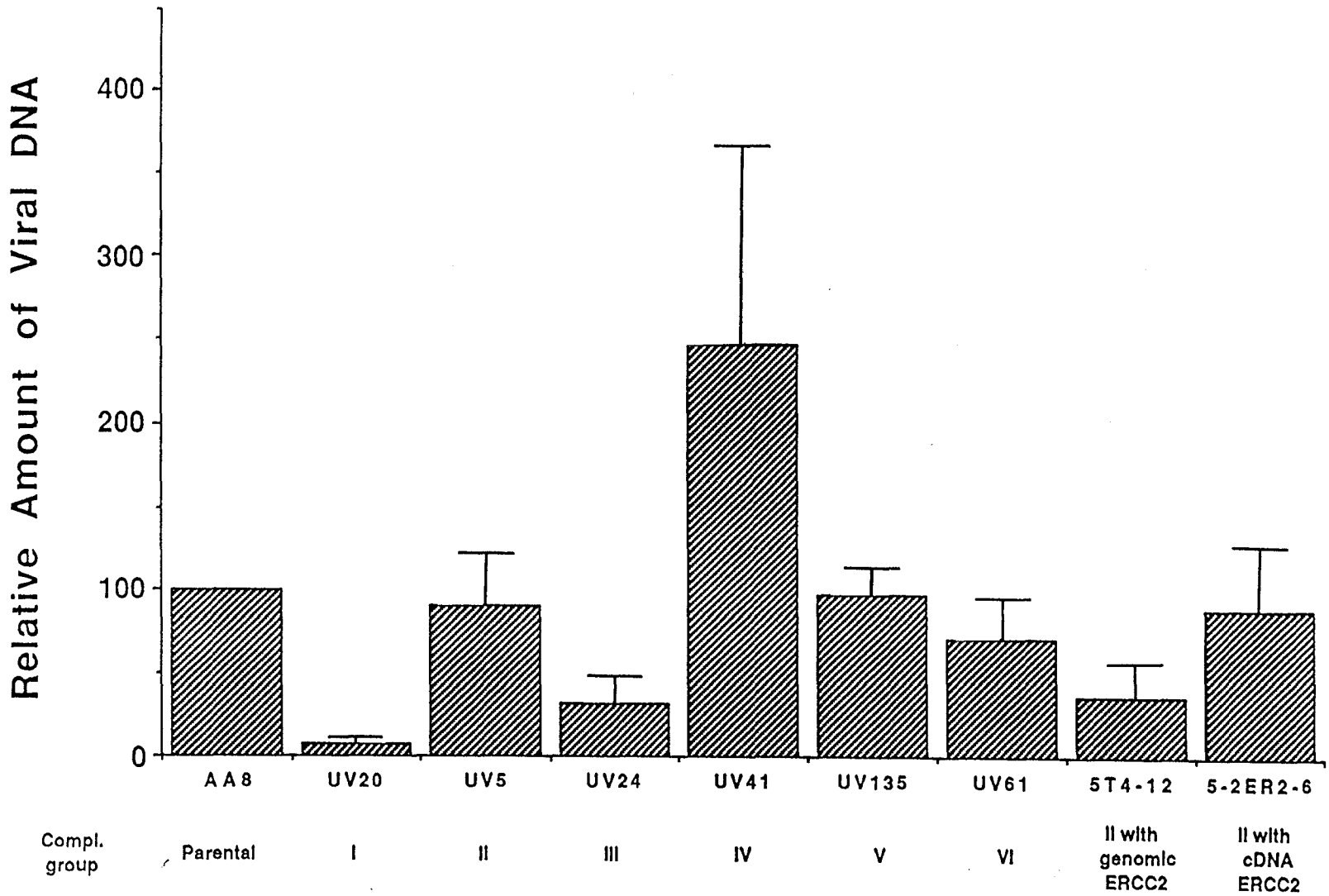

Relative Amount of Viral DNA
400
300
200
100
0
AA8
UV20
UV5
UV24
UV41
UV135
UV61
5T4-12
5-2ER2-6
Compl. group
Parental
I
II
III
IV
V
VI
II with genomic ERCC2
II with cDNA ERCC2

Figure 32: Histogram of relative viral DNA synthesis of AA8 and its derived cells following infection with unirradiated Ad5(LacZ)

In individual experiments, which included AA8 and at least two mutant lines, cells were infected with unirradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 72 hours post-infection for each cell line, relative to AA8. The mean relative viral DNA synthesis of each cell line for a number of experiments, as indicated in Table 11, was calculated and is presented in this figure. Error bars are standard error.

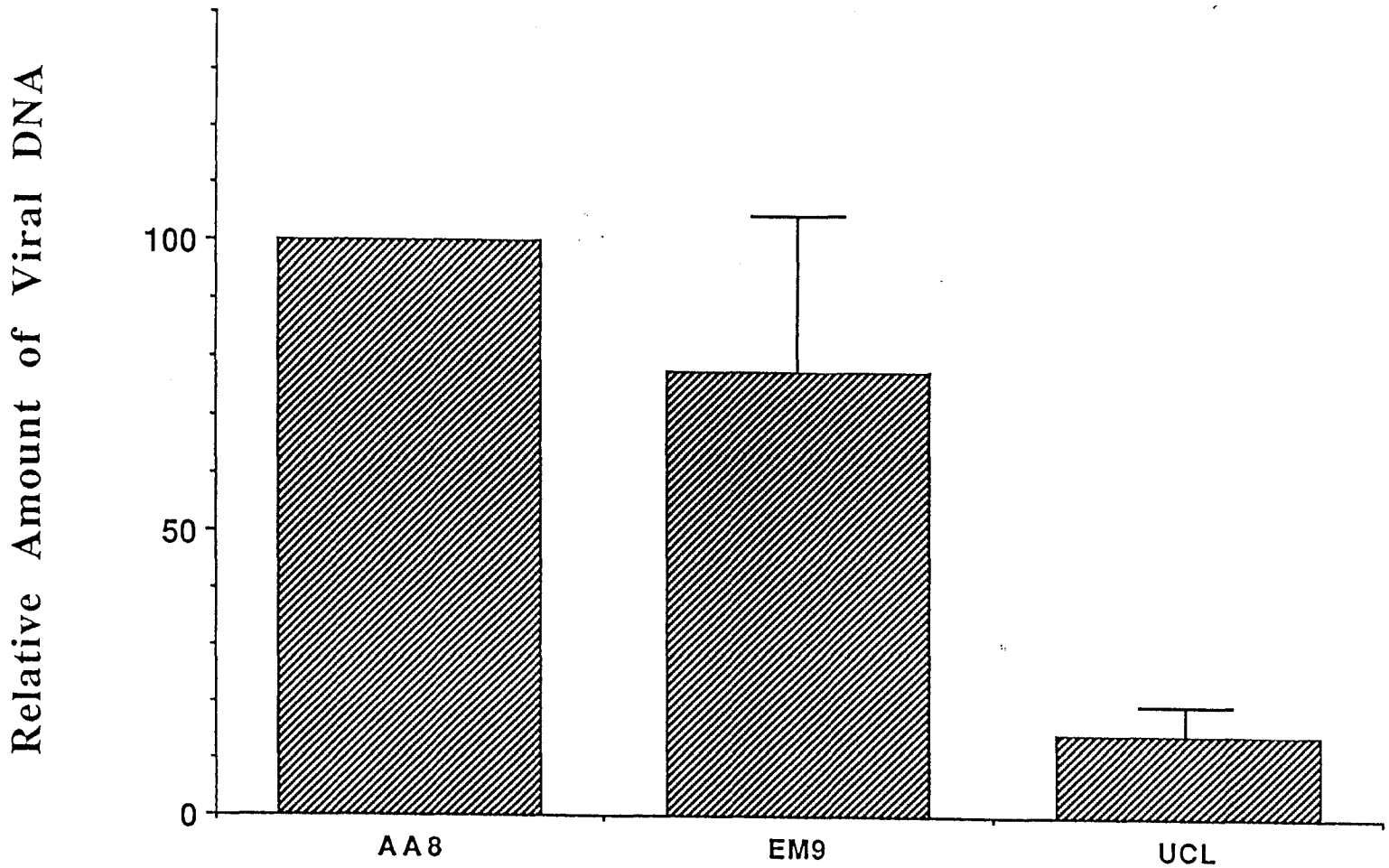

Relative Amount of Viral DNA
100
50
0
AA8
EM9
UCL

Figure 33: Histogram of relative viral DNA synthesis of K1 and its derived UV-sensitive cells following infection with unirradiated Ad5(LacZ)

In individual experiments, which included K1 and at least two mutant lines, cells were infected with unirradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 72 hours post-infection for each cell line, relative to K1. The mean relative viral DNA synthesis of each cell line for a number of experiments, as indicated in Table 11, was calculated and is presented in this figure. Error bars are standard error.

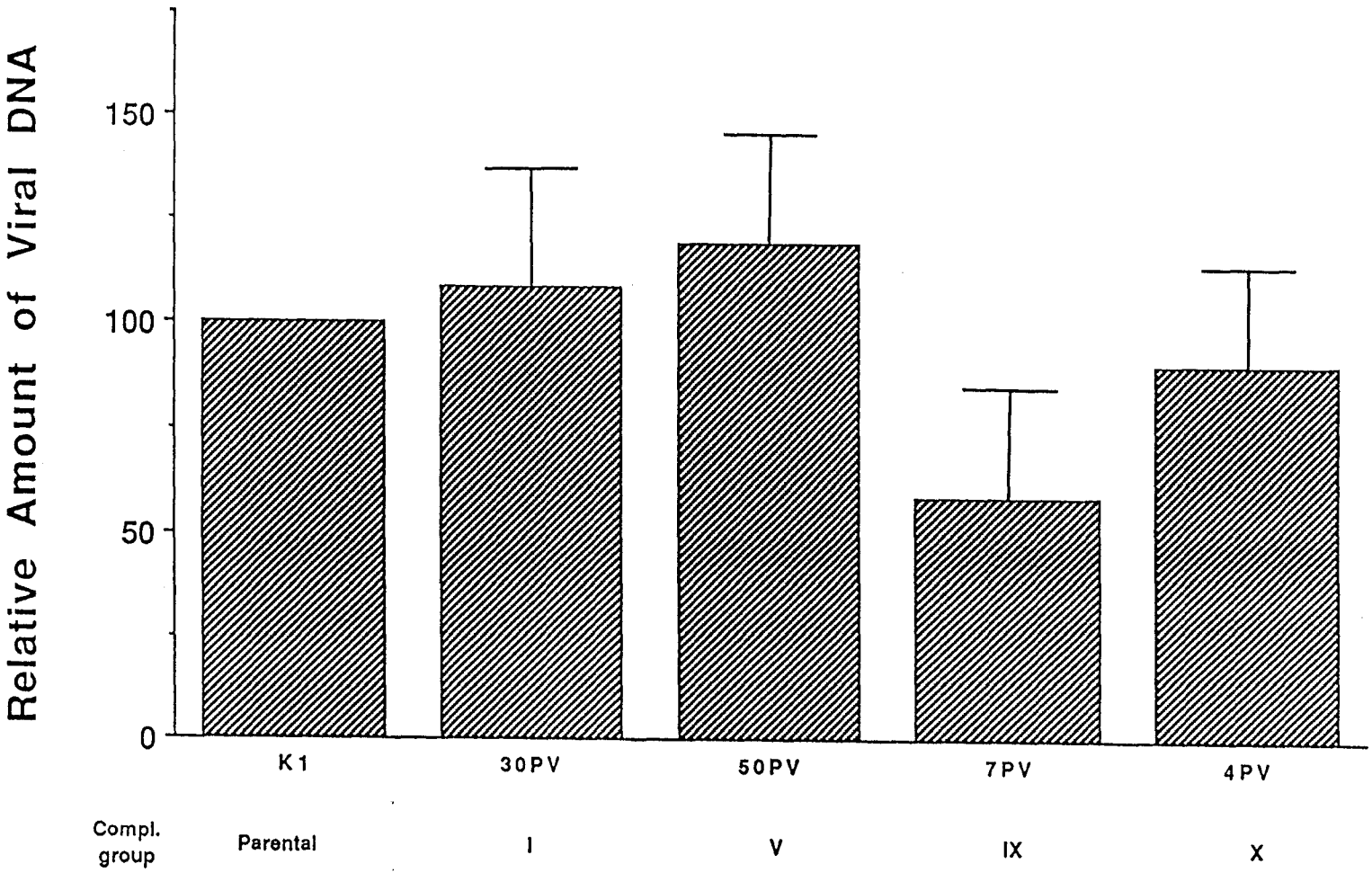
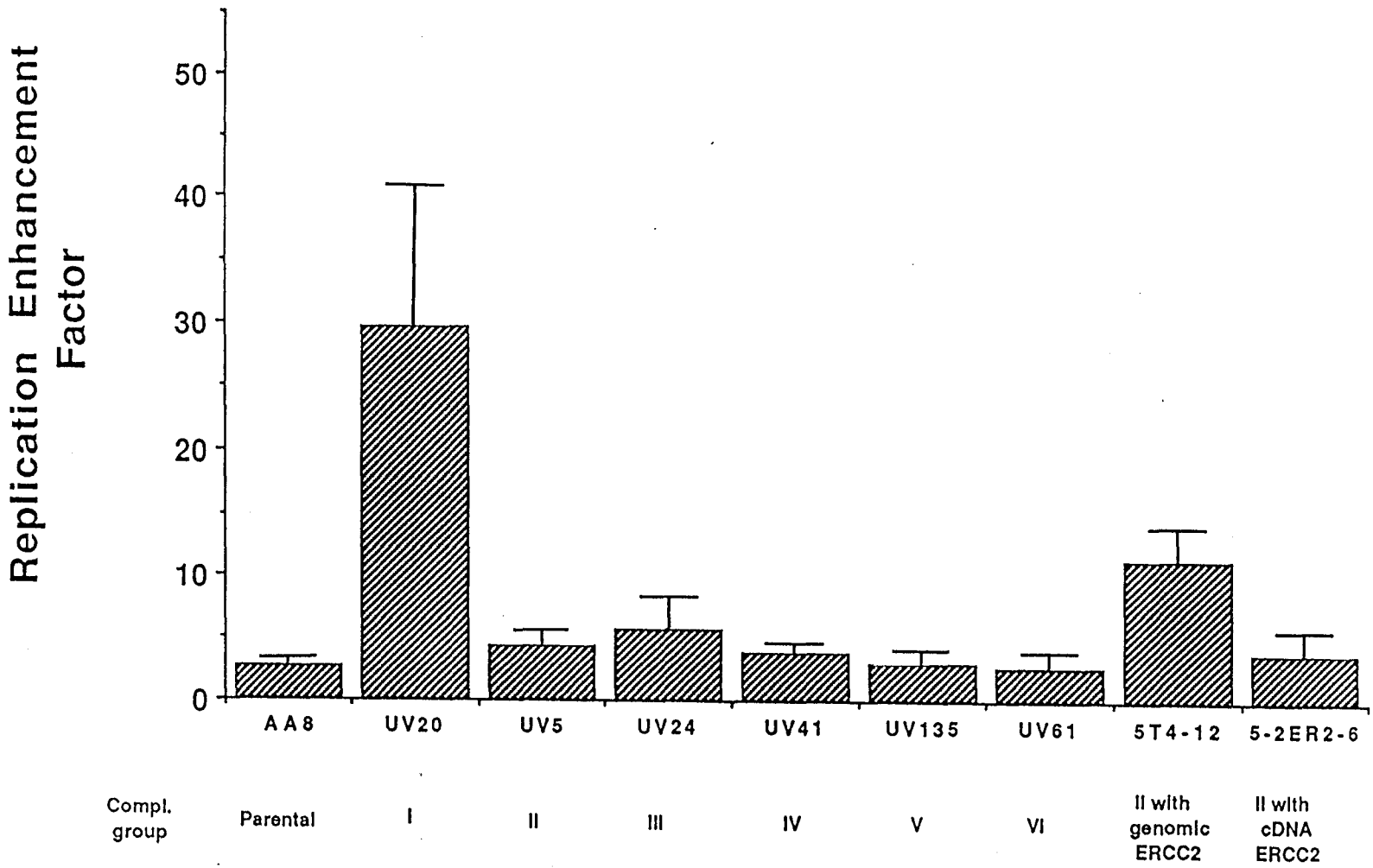

Relative Amount of Viral DNA
150
100
50
0
K1
30PV
50PV
7PV
4PV
Compl. group
Parental
I
V
IX
X

Figure 34: Histogram of viral DNA replication enhancement following infection of AA8 and its derived UV-sensitive cells with Ad5(denV)

In individual experiments, which included AA8 and at least two mutant lines, cells were infected with unirradiated Ad5(denV) or unirradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 72 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(denV) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(denV) REF of each cell line for a number of experiments, as indicated in Table 13, was calculated and is presented in this figure. Error bars are standard error.


Replication Enhancement Factor
50
40
30
20
10
0
AA8
UV20
UV5
UV24
UV41
UV135
UV61
5T4-12
5-2ER2-6
Compl. group
Parental
I
II
III
IV
V
VI
II with genomic ERCC2
II with cDNA ERCC2

further examine the replication of Ad5(denV) DNA compared to that of Ad5(LacZ) in CHO cells.

Each experiment performed included a parental cell line and at least two mutant cell lines. Cells were infected with non-irradiated Ad5(denV) or Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA extracted from the cells at 4 hours p.i. and 72 hours p.i. Quantification of viral DNA was done by slot-blotting, hybridization to a radioactive Ad DNA probe and scintillation counting. The net amount of viral DNA synthesized by 72 hours p.i. was determined by subtracting the amount of viral DNA at 4 hours p.i. from the quantity found at 72 hours p.i.

The effect of the *denV* gene on viral DNA replication in CHO cells was assessed by calculating the replication enhancement factor (REF). The REF is defined as the amount of Ad5(denV) replication in a cell line relative to that of Ad5(LacZ) in the same cell line for a particular experiment. The mean REF from a number of experiments, as indicated in Table 13, was then calculated along with the associated standard deviation and presented in Figure 34. AA8 and all of its derived UV-sensitive mutants, including the *ERCC2*-tranfected UV5 mutants demonstrated Ad5(denV) REFs significantly above 1 (Table 13). The largest effect was seen in UV20 cells which exhibited an Ad5(denV) REF of 29.75 $\pm$ 12.57. The REF for Ad5(denV) DNA synthesis was also increased significantly for EM9 and UCL (Figure 35).

Significant increases in the Ad5(denV) REFs were demonstrated for K1 and all its derived UV-sensitive PV mutants (Figure 36). The Ad5(denV) REF for 7PV was the largest of this group and was determined to be 27.72 $\pm$ 5.29 (Table

Figure 35: Histogram of viral DNA replication enhancement following infection of AA8 and its derived cells with Ad5(denV)

In individual experiments, which included AA8 and at least two mutant lines, cells were infected with unirradiated Ad5(denV) or unirradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 72 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(denV) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(denV) REF of each cell line for a number of experiments, as indicated in Table 13, was calculated and is presented in this figure. Error bars are standard error.

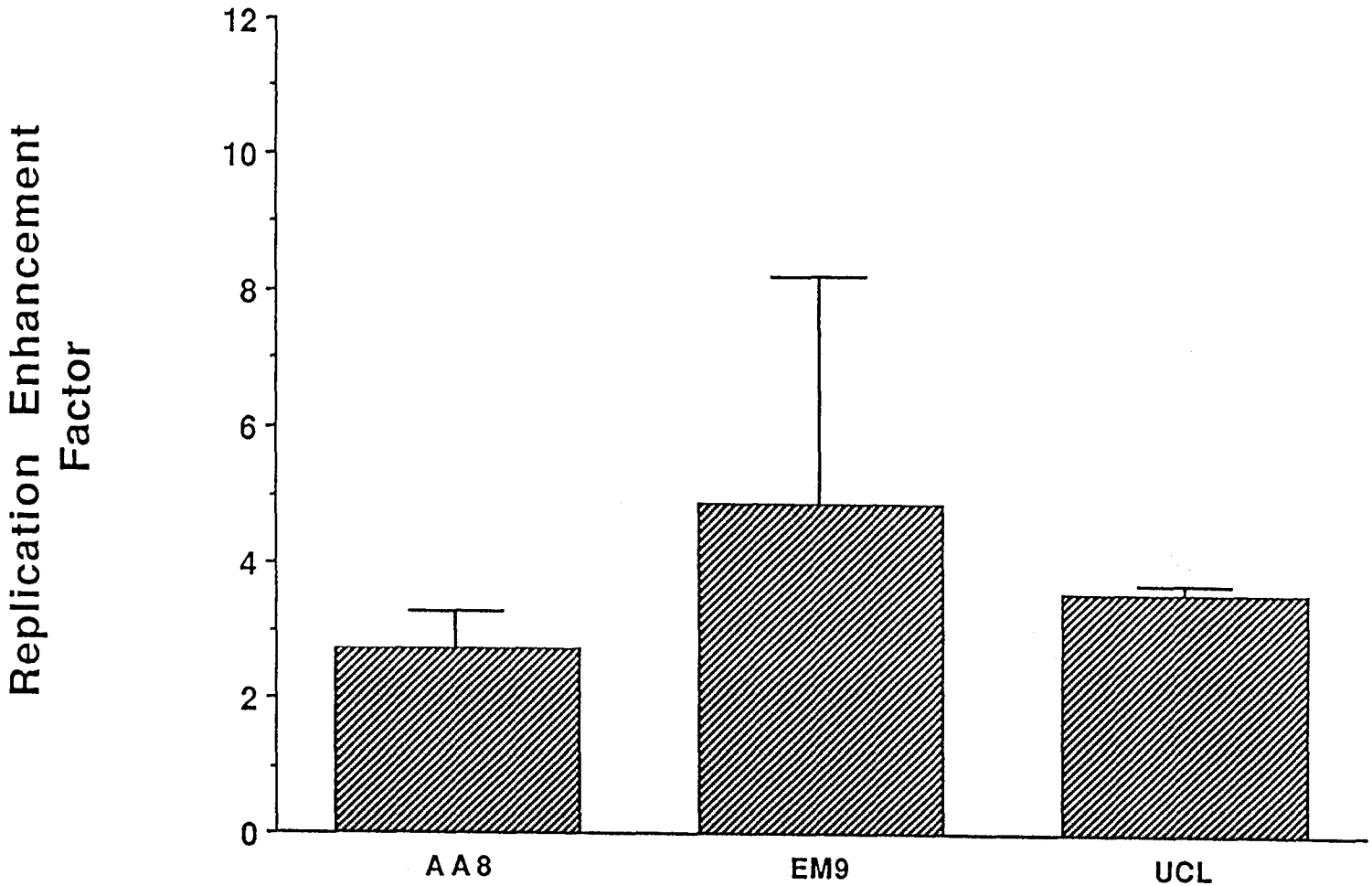
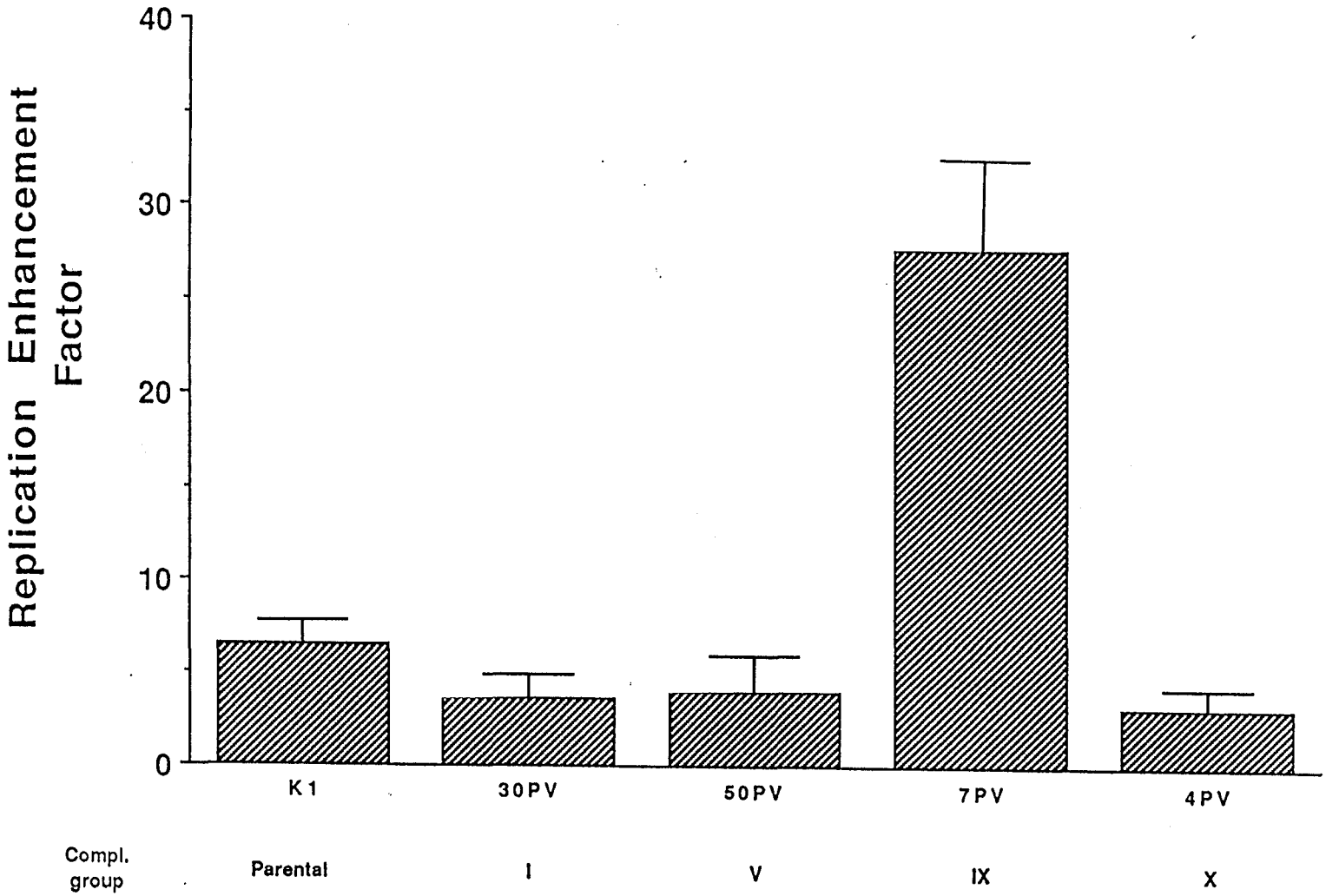

Replication Enhancement Factor
12
10
8
6
4
2
0
AA8
EM9
UCL

Figure 36: Histogram of viral DNA replication enhancement following infection of K1 and its derived UV-sensitive cells with Ad5(denV)

In individual experiments, which included K1 and at least two mutant lines, cells were infected with unirradiated Ad5(denV) or unirradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 72 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(denV) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(denV) REF of each cell line for a number of experiments, as indicated in Table 14, was calculated and is presented in this figure. Error bars are standard error.


Replication Enhancement Factor
40
30
20
10
0
K1
30PV
50PV
7PV
4PV
Compl. group
Parental
I
V
IX
X

14). This large enhancement in replication of Ad5(denV) DNA compared to that of Ad5(LacZ) in 7PV is similar to the large increase seen in UV20 (Table 13).

C.3. Replication of Ad5(ERCC1) DNA in Normal and Repair-Deficient CHO Cells

Two DNA replication proteins, human single-stranded DNA binding protein (hssb) and proliferating cell nuclear antigen (PCNA), have recently been shown to also be involved in the process of DNA excision repair (Coverley *et al.* 1991; Shivji *et al.* 1992). Initial experiments in this study indicated that replication of non-irradiated Ad5(ERCC1) DNA may be enhanced compared to that of Ad5(LacZ) in CHO cells. It was therefore considered to be of interest to further examine the DNA replication of Ad5(ERCC1) compared to Ad5(LacZ) in CHO cells to investigate the possibility that the DNA repair gene, *ERCC1*, also plays a role in DNA replication.

Each experiment performed included a parental cell line and at least two mutants cell lines. Cells were infected with non-irradiated Ad5(ERCC1) or Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA extracted from the cells at 4 hours p.i. and 72 hours p.i. Quantification of viral DNA was done by slot-blotting, hybridization to a radioactive Ad DNA probe and scintillation counting. The net amount of viral DNA synthesized by 72 hours p.i. was determined by subtracting the amount of viral DNA at 4 hours p.i. from the quantity found at 72 hours p.i.

The replication of Ad5(ERCC1) DNA was significantly enhanced compared to that of Ad5(LacZ) for AA8 and all of its derived UV-sensitive

mutants examined (Figure 37). UV20 was found to exhibit the largest increase in Ad5(ERCC1) DNA replication with a REF of 19.88 ± 5.55 (Table 13). The increased Ad5(ERCC1) replication in the other cell lines was seen to be in the order of 3- to 8-fold.

UCL exhibited a significant increase in its Ad5(ERCC1) REF as did the EMS-sensitive EM9 cell line (Figure 38). The Ad5(ERCC1) REFs for UCL and EM9 are summarized in Table 13.

The REFs for Ad5(ERCC1) in K1, 30PV, 50PV, 7PV, and 4PV were significantly increased above 1 (Figure 39). As for Ad5(denV) REFs, 7PV demonstrates a large enhancement of Ad5(ERCC1) DNA replication with a REF of 23.48 ± 7.29 (Table 14).

C.4. Replication of Ad5(LacZ) DNA in Human Cells

Human fibroblasts and human transformed cells were infected with non-irradiated Ad5(LacZ) at a MOI of 200 PFU/cell and 40 PFU/cell, respectively. The viral DNA was extracted from the cells at 4 hours p.i. and 36 hours p.i. and quantified by slot-blotting, hybridization to a radioactive Ad DNA probe and scintillation counting. The net amount of viral DNA synthesized by 36 hours p.i. was determined by subtracting the amount of viral DNA at 4 hours p.i. from the quantity found at 36 hours p.i. Uptake of virus by each cell line in individual experiments was assessed by examining the relative amounts of viral DNA scored at the 4 hour p.i. time points. This method of assessment of viral uptake did not reveal any differences between the fibroblasts nor did it reveal any differences among the transformed cell lines.

Figure 37: Histogram of viral DNA replication enhancement following infection of AA8 and its derived UV-sensitive cells with Ad5(ERCC1)

In individual experiments, which included AA8 and at least two mutant lines, cells were infected with non-irradiated Ad5(ERCC1) or non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 72 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(ERCC1) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(ERCC1) REF of each cell line for a number of experiments, as indicated in Table 13, was calculated and is presented in this figure. Error bars are standard error.

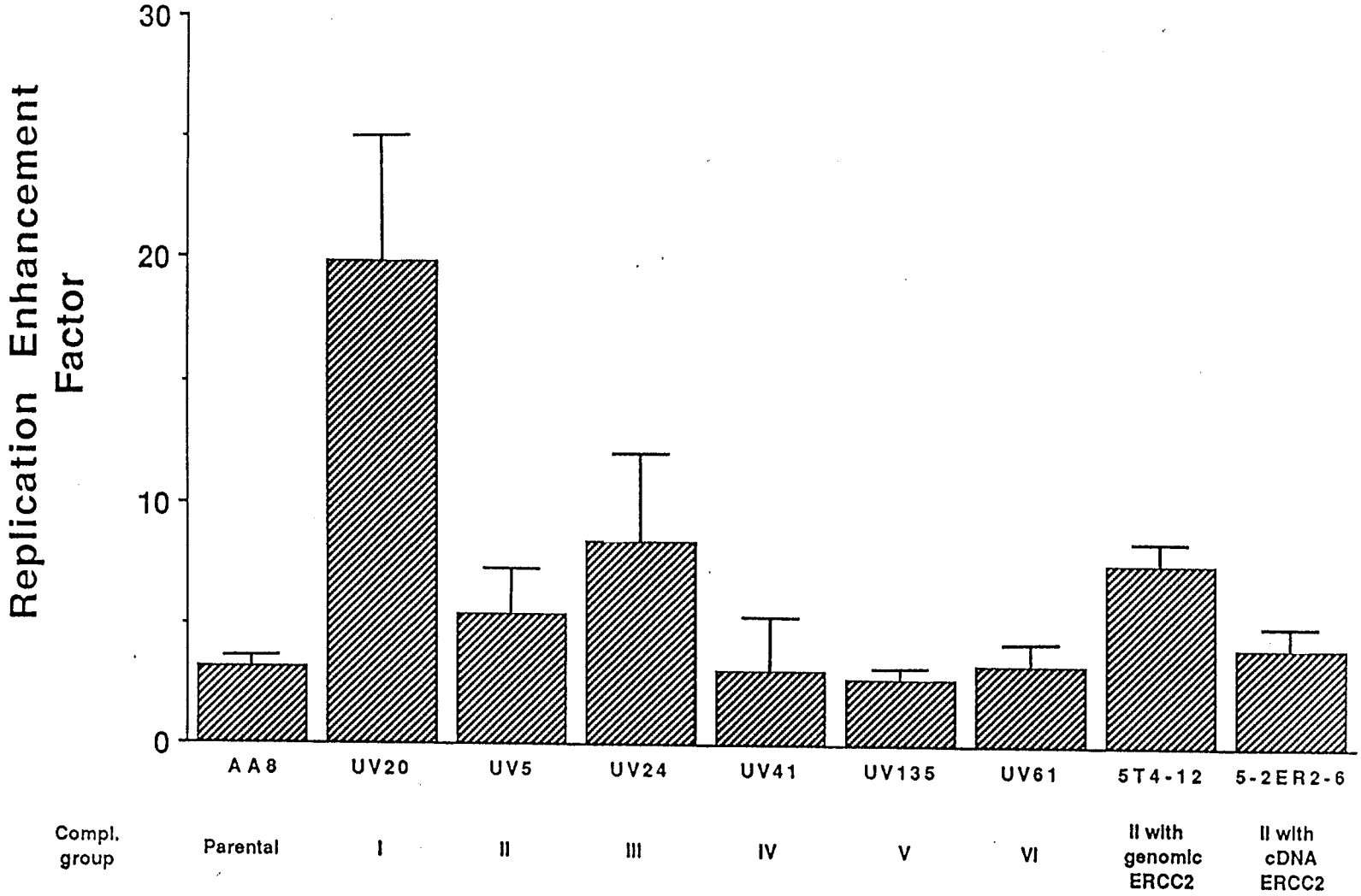

Replication Enhancement Factor
30
20
10
0
AA8
UV20
UV5
UV24
UV41
UV135
UV61
5T4-12
5-2ER2-6
Compl. group
Parental
I
II
III
IV
V
VI
II with genomic ERCC2
II with cDNA ERCC2

Figure 38: Histogram of viral DNA replication enhancement following infection of AA8 and its derived cells with Ad5(ERCC1)

In individual experiments, which included AA8 and at least two mutant lines, cells were infected with non-irradiated Ad5(ERCC1) or non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 72 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(ERCC1) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(ERCC1) REF of each cell line for a number of experiments, as indicated in Table 13, was calculated and is presented in this figure. Error bars are standard error.

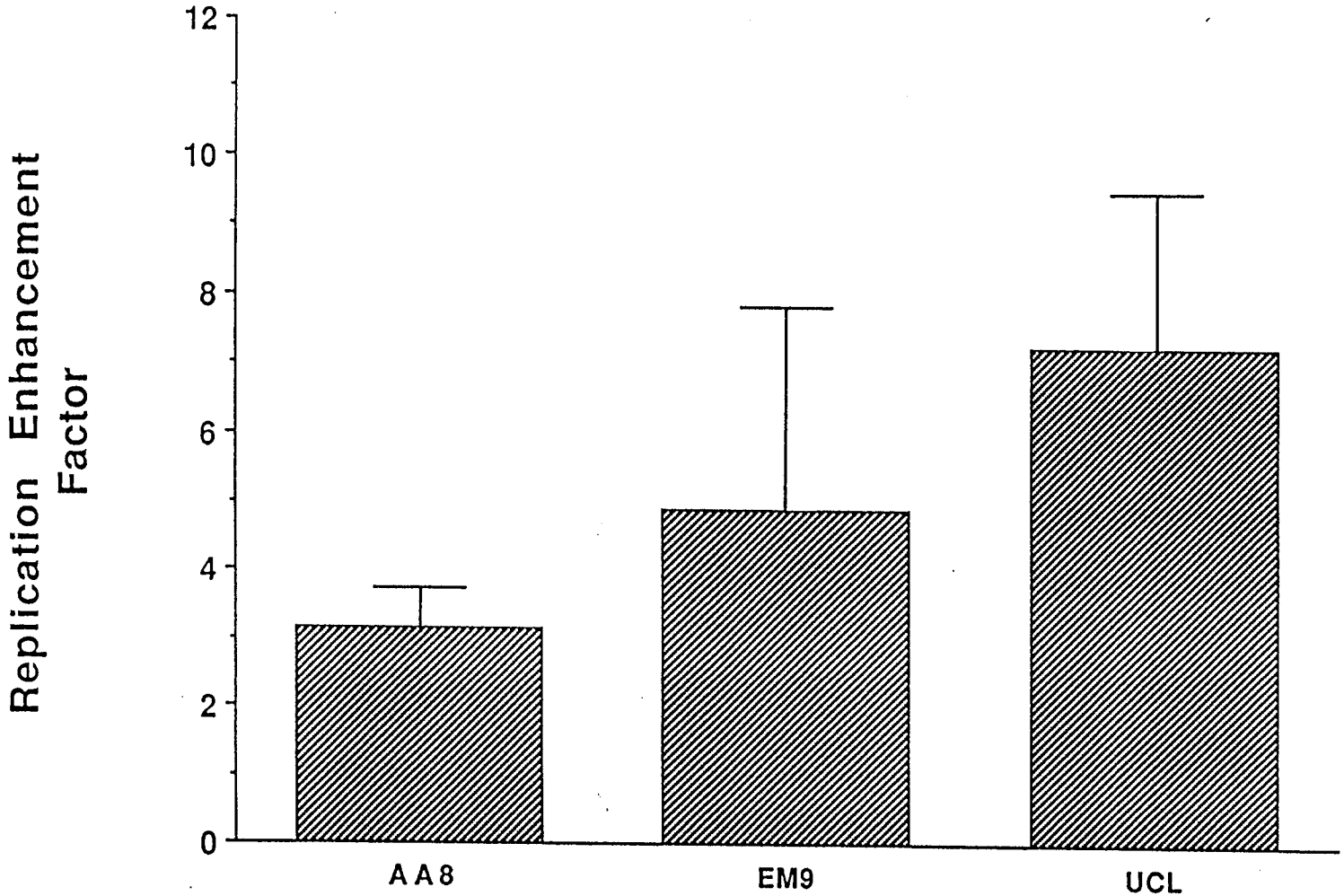
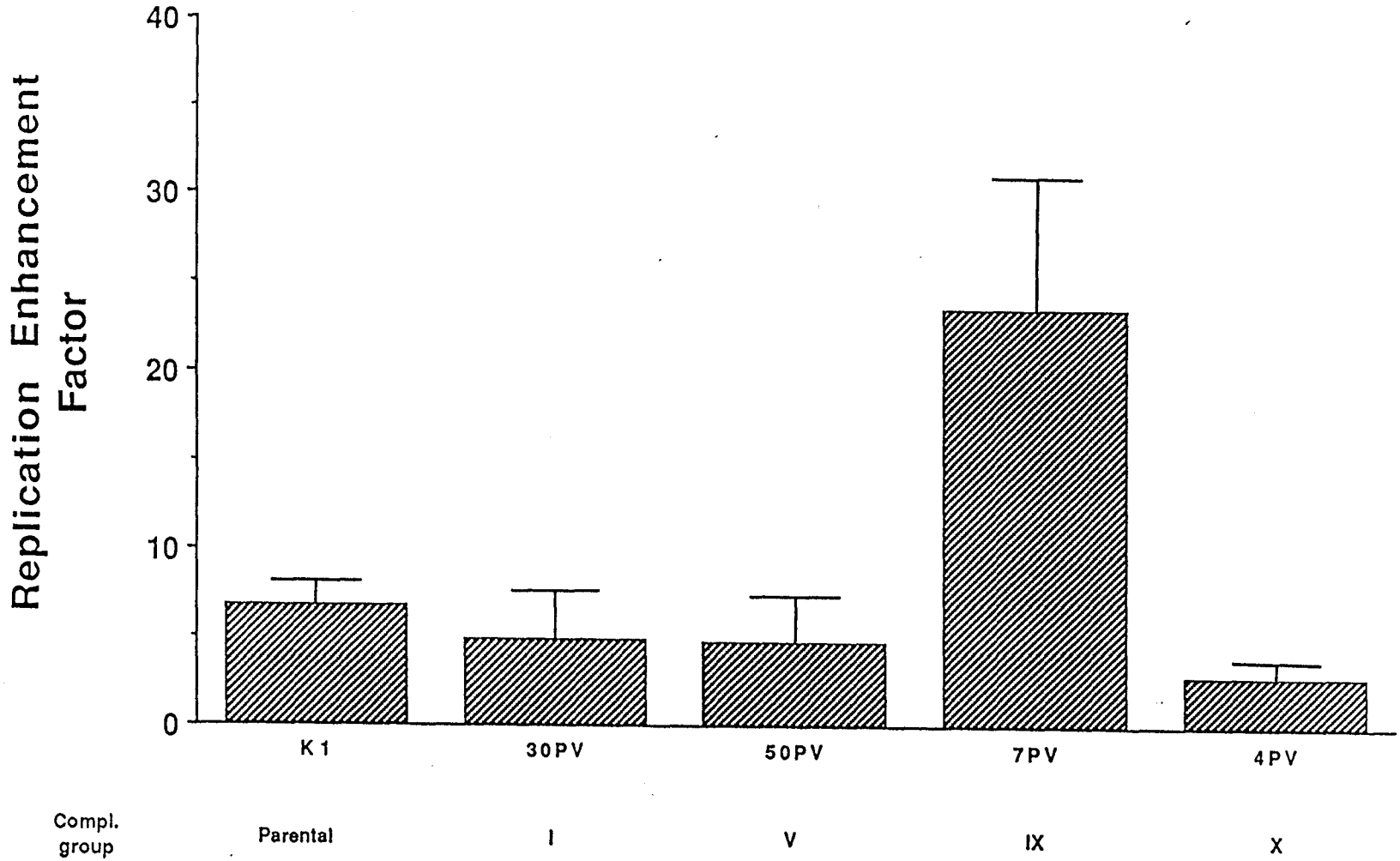

Replication Enhancement Factor
12
10
8
6
4
2
0
AA8
EM9
UCL

Figure 39: Histogram of viral DNA replication enhancement following infection of K1 and its derived UV-sensitive cells with Ad5(ERCC1)

In individual experiments, which included K1 and at least two mutant lines, cells were infected with non-irradiated Ad5(ERCC1) or non-irradiated Ad5(LacZ) at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 72 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(ERCC1) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(ERCC1) REF of each cell line for a number of experiments, as indicated in Table 14, was calculated and is presented in this figure. Error bars are standard error.


Replication Enhancement Factor
40
30
20
10
0
K 1
30PV
50PV
7PV
4PV
Compl. group
Parental
I
V
IX
X

The amount of Ad5(LacZ) DNA replicated in XP1BR group D cells by 36 hours p.i. was found to be significantly reduced to 44.62 ± 4.18% compared to the amounts replicated in normal GM969 fibroblasts (Table 15; Figure 40).

Replication of Ad5(LacZ) DNA in HeLa cells was not found to be significantly different from that in the apparently repair-proficient 293 cells while Ad5(LacZ) DNA replication in HT29 cells was significantly reduced to 20.70 ± 9.07% of 293 values (Figure 41; Table 15).

C.5. Replication of Ad5(denV) DNA in Human Cells

As some initial results suggested an enhancement of viral DNA replication in CHO cells by *denV*, it was considered of interest to examine the possibility of such an enhancement in human cells.

Human fibroblasts and human transformed cells were infected with non-irradiated Ad5(denV) at a MOI of 200 PFU/cell and 40 PFU/cell, respectively. The viral DNA was extracted from the cells at 4 hours p.i. and 36 hours p.i. and quantified by slot-blotting, hybridization to a radioactive Ad DNA probe and scintillation counting. The net amount of viral DNA synthesized by 36 hours p.i. was determined by subtracting the amount of viral DNA at 4 hours p.i. from thequantity found at 36 hours p.i.

The replication of Ad5(denV) in human fibroblasts and transformed human cells was compared to that of Ad5(LacZ) in these same cells to assess the role of the denV protein in viral DNA replication. A REF, the ratio of Ad5(denV) DNA synthesis at 36 hours p.i. in a cell line compared to that of Ad5(LacZ) infected cells, was used to measure the effect of the denV protein.

Figure 40: Replication of viral DNA in GM969 (normal) cells and GM3615 (XP1BR group D) following infection with unirradiated Ad5(LacZ)

In individual experiments, which included GM969 and XP1BR group D fibroblasts, cells were infected with unirradiated Ad5(LacZ) at a MOI of 200 PFU/cell and viral DNA synthesis was scored at 36 hours post-infection for each cell stain, relative to GM969. The mean relative viral DNA synthesis of each cell line for a number of experiments, as indicated in Table 15, was calculated and is presented in this figure. Error bars are standard error.

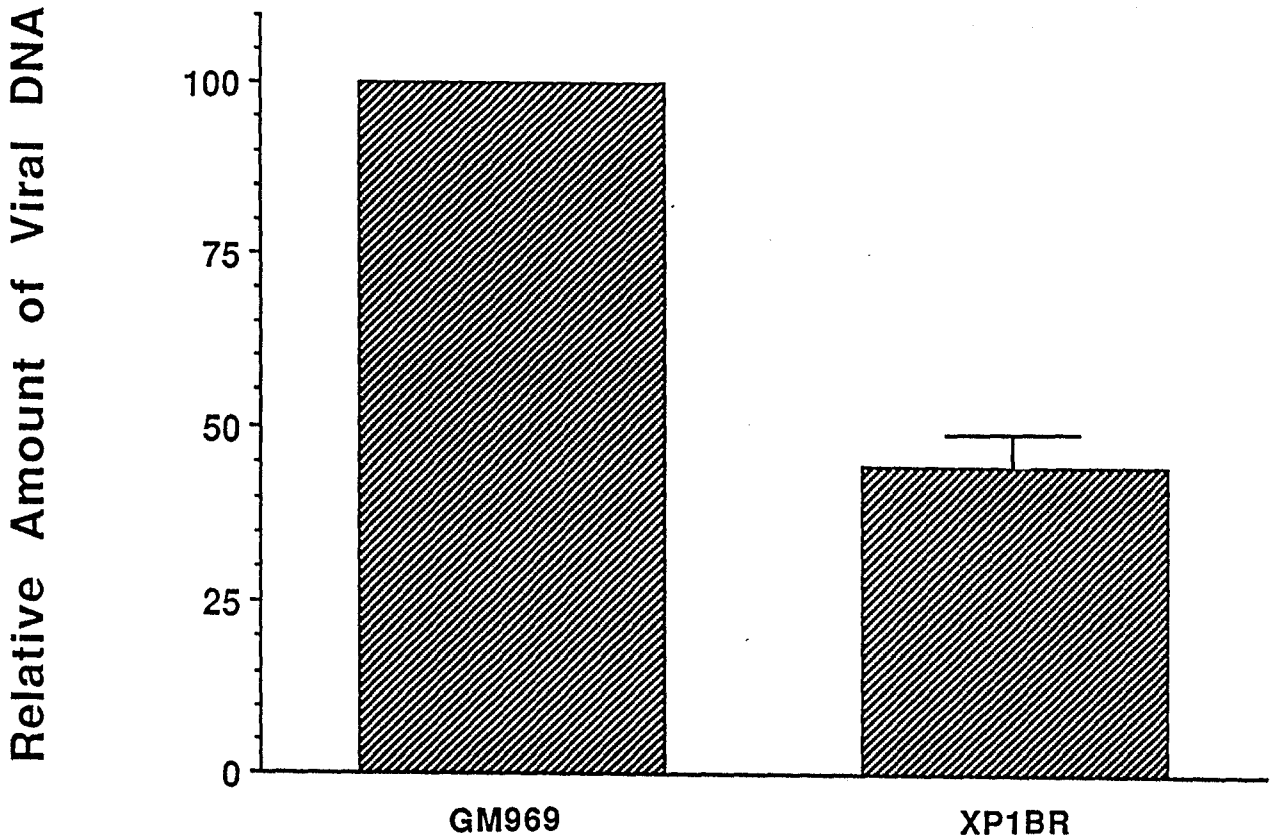
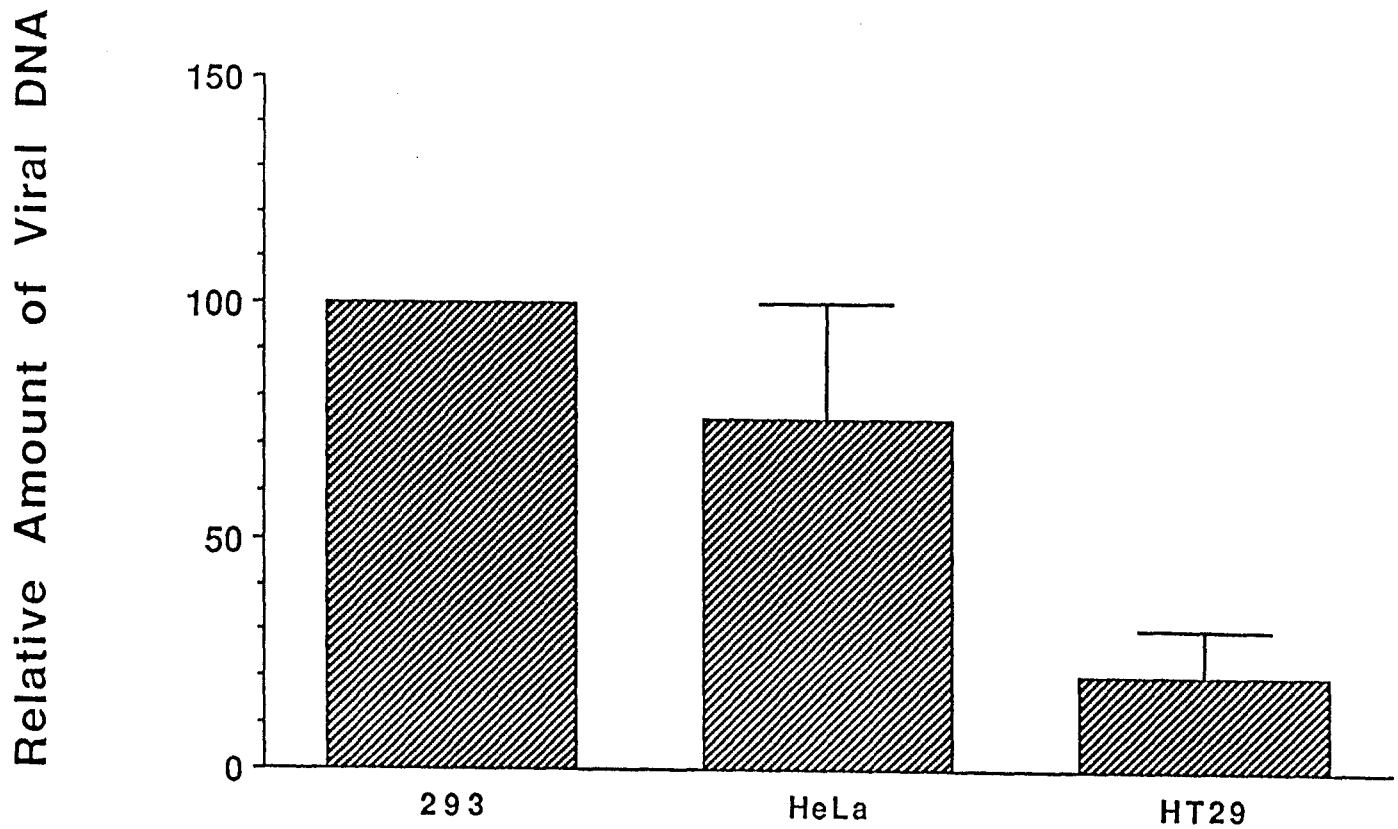

Relative Amount of Viral DNA
100
75
50
25
0
GM969
XP1BR

Figure 41: Replication of viral DNA in 293, HeLa, and HT29 cells following infection with unirradiated Ad5(LacZ)

In individual experiments, which included 293 cells and HeLa and/or HT29 cells, infection with unirradiated Ad5(LacZ) was carried out at a MOI of 40 PFU/cell. Viral DNA synthesis was scored at 36 hours post-infection for each cell line, relative to 293. The mean relative viral DNA synthesis of each cell line for a number of experiments, as indicated in Table 15, was calculated and is presented in this figure. Error bars are standard error.


Relative Amount of Viral DNA
150
100
50
0
293
HeLa
HT29

Ad5(denV) DNA replication was found to be significantly increased compared to Ad5(LacZ) DNA replication for both normal GM969 and XP1BR group D fibroblasts (Figure 42). The Ad5(denV) REF for normal GM969 was determined to be 1.65 ± 0.02 which is less than the values found for normal CHO cells (Table 13; Table 16). XP1BR fibroblasts exhibited an Ad5(denV) REF of 3.76 ± 1.37 which is comparable to that of most CHO cells which exhibit enhanced Ad5(denV) DNA replication, with the exception of UV20 and 7PV which are enhanced to a much greater extent.

Infection of 293 cells with Ad5(denV) resulted in a significant decrease in viral DNA synthesis compared to Ad5(LacZ) (Figure 43). The Ad5(denV) REF for 293 was 0.88 ± 0.07 (Table 16). HeLa cells demonstrated a significant Ad5(denV) REF of 1.41 ± 0.19 (Figure 43; Table 16). The effect of the *denV* gene on viral DNA replication in transformed human cells is much less pronounced than in human fibroblasts or CHO cells.

C.6. Replication of Ad5(ERCC1) DNA Human Cells

The demonstration that hssb and PCNA are both involved in DNA repair and DNA replication coupled with initial experiments in this study which raised the possibility of an involvement of ERCC1 in viral DNA replication lead to a further examination of Ad5(ERCC1) DNA replication in human cells.

Human fibroblasts and human transformed cells were infected with non-irradiated Ad5(denV) at a MOI of 200 PFU/cell and 40 PFU/cell, respectively. The viral DNA was extracted from the cells at 4 hours p.i. and 36 hours p.i. and quantified by slot-blotting, hybridization to a radioactive Ad DNA probe and

Figure 42: Histogram of viral DNA replication enhancement following infection of GM969 (normal) cells and GM3615 (XP1BR group D) cells with Ad5(denV)

In individual experiments, which included GM969 and XP1BR group D fibroblasts, cells were infected with unirradiated Ad5(denV) or Ad5(LacZ) at a MOI of 200 PFU/cell and viral DNA synthesis was scored at 36 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(denV) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(denV) REF of each cell line for a number of experiments, as indicated in Table 16, was calculated and is presented in this figure. Error bars are standard error.

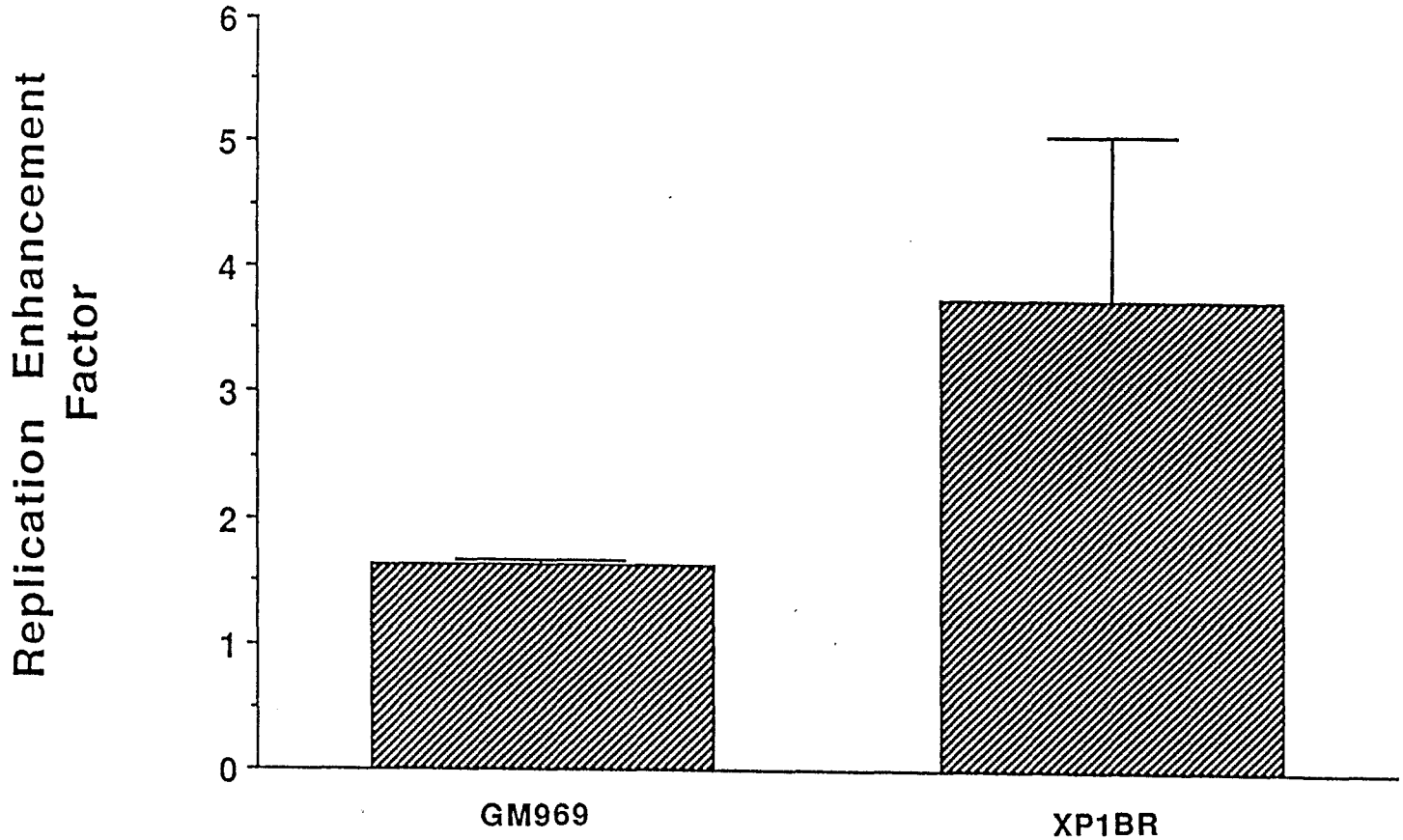
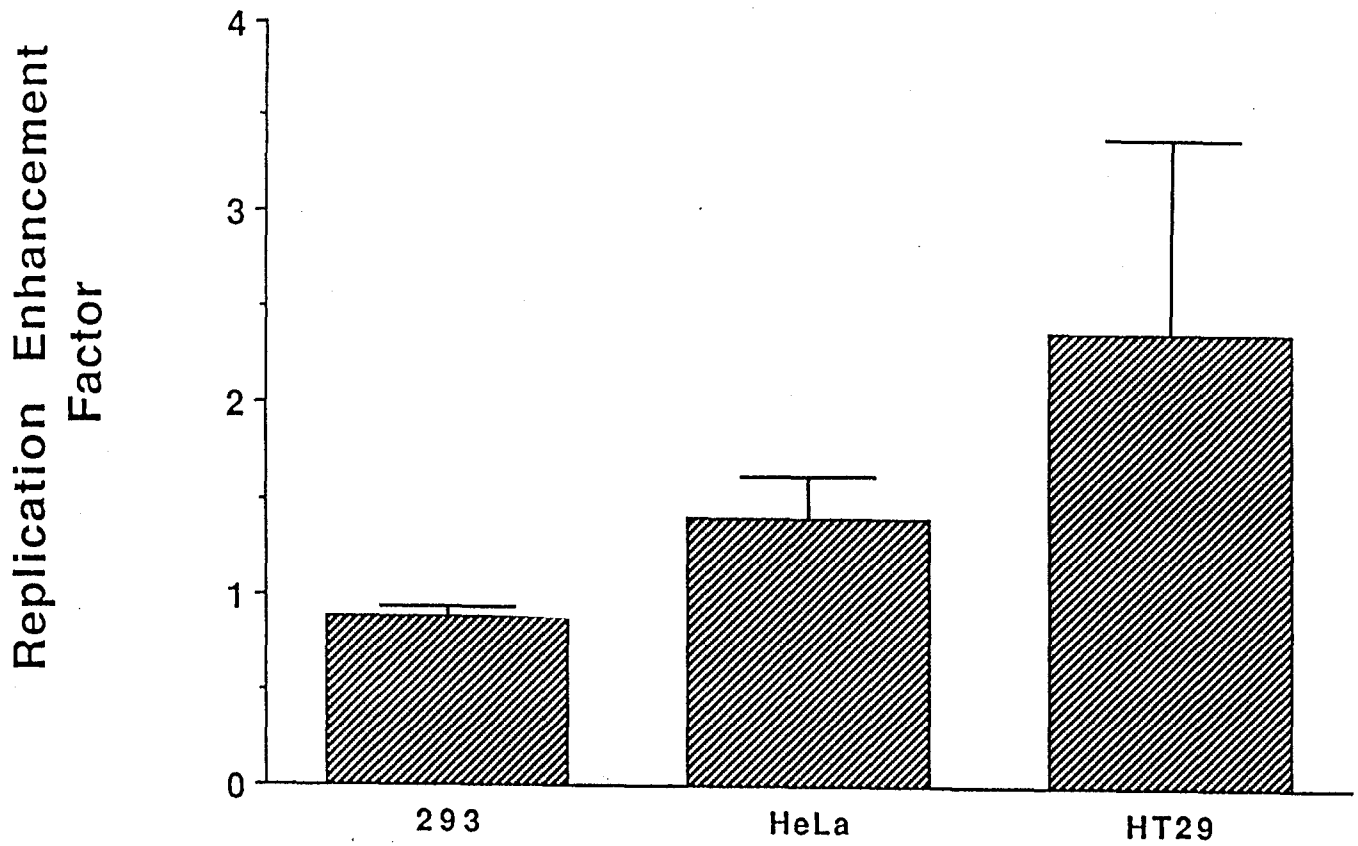

Replication Enhancement Factor
6
5
4
3
2
1
0
GM969
XP1BR

Figure 43: Histogram of viral DNA replication enhancement following infection of 293, HeLa, and HT29 cells with Ad5(denV)

In individual experiments, which included 293 and HeLa and/or HT29 cells, infection with unirradiated Ad5(denV) or Ad5(LacZ) was carried out at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 36 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(denV) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(denV) REF of each cell line for a number of experiments, as indicated in Table 16, was calculated and is presented in this figure. Error bars are standard error.


Replication Enhancement Factor
4
3
2
1
0
293
HeLa
HT29

scintillation counting. The net amount of viral DNA synthesized by 36 hours p.i. was determined by subtracting the amount of viral DNA at 4 hours p.i. from the quantity found at 36 hours p.i.

Normal GM969 fibroblasts are not significantly altered in the amounts of viral DNA synthesized by 36 hours p.i. following infection with Ad5(ERCC1) compared to Ad5(LacZ) infection (Figure 44). The Ad5(ERCC1) REF for GM969 was determined to be 1.33 $\pm$ 0.59 (Table 16). However, the repair-deficient XP1BR group D cells did demonstrate a significant increase of 2.94 $\pm$ 1.10 in Ad5(ERCC1) REF (Table 16).

Interestingly, the Ad5(ERCC1) REF in 293 cells was found to be significantly reduced (Figure 45). This is the only cell type examined, including transformed human cells, human fibroblasts, and CHO cells which is seen to be hindered by *ERCC1* in terms of viral DNA replication. Infection of HeLa cells with Ad5(ERCC1) does not increase viral DNA synthesis compared to Ad5(LacZ) while HT29 exhibits an increase in its Ad5(ERCC1) REF of 2.22 $\pm$ 0.72 (Table 16) which is smaller than most Ad5(ERCC1) REFs in CHO cell lines.

D. Adenoviral DNA Replication in Human, Hamster, and Murine Cells

Differences in viral DNA replication among cell lines as well as among the recombinant viruses have been established in this study. However, these differences were determined by scoring at 72 hours p.i. for CHO cells and at 36 hours p.i. for human cells. It was therefore considered of interest to examine in more detail the time course of viral DNA synthesis of the 3 recombinant viruses

Figure 44: Histogram of viral DNA replication enhancement following infection of N969b (normal) cells and GM3615 (XP1BR group D) cells with Ad5(ERCC1)

In individual experiments, which included GM969 and XP1BR group D fibroblasts, infection with unirradiated Ad5(denV) or Ad5(LacZ) was carried out at a MOI of 200 PFU/cell and viral DNA synthesis was scored at 36 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(denV) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(denV) REF of each cell line for a number of experiments, as indicated in Table 16, was calculated and is presented in this figure. Error bars are standard error.

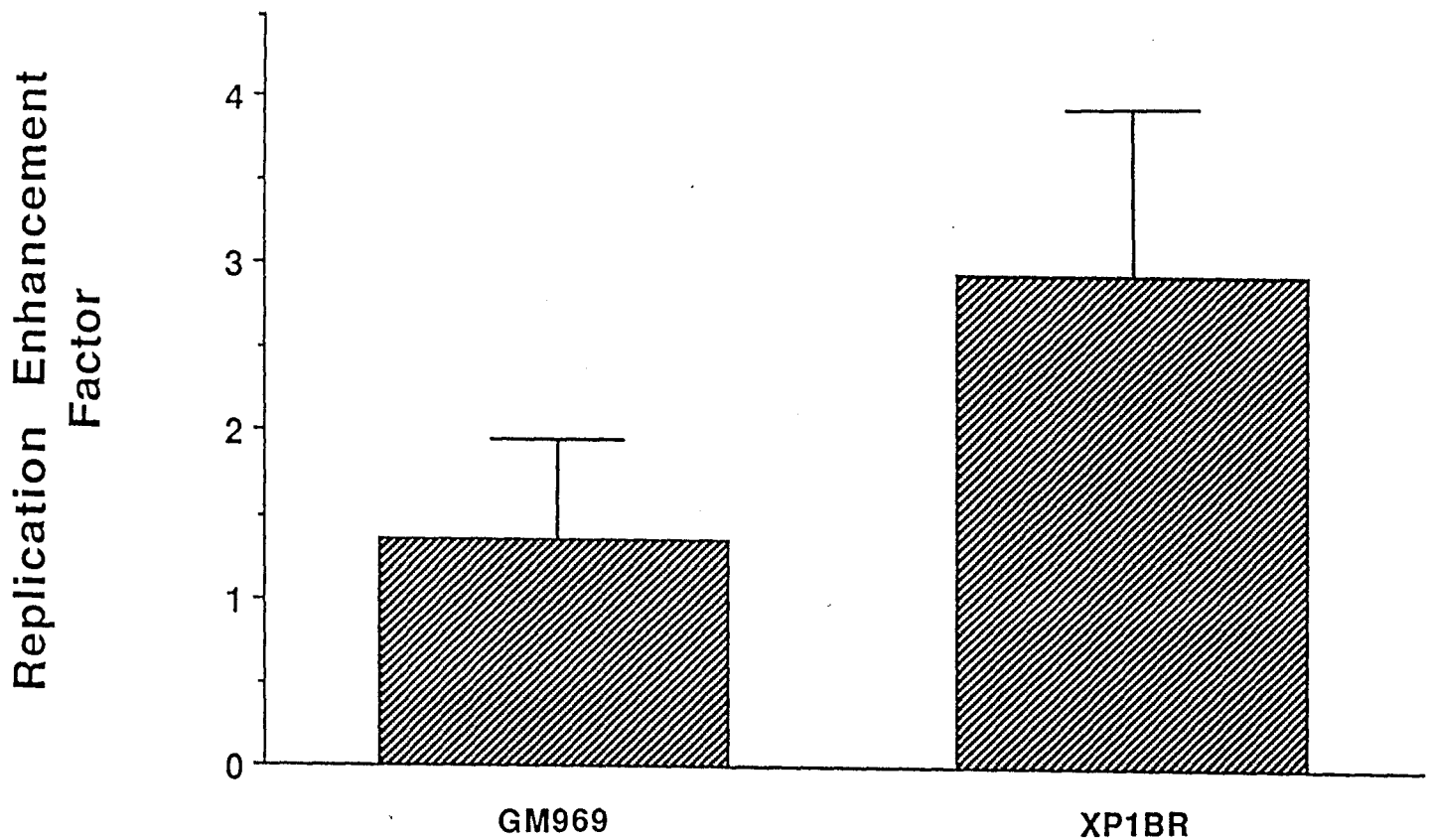
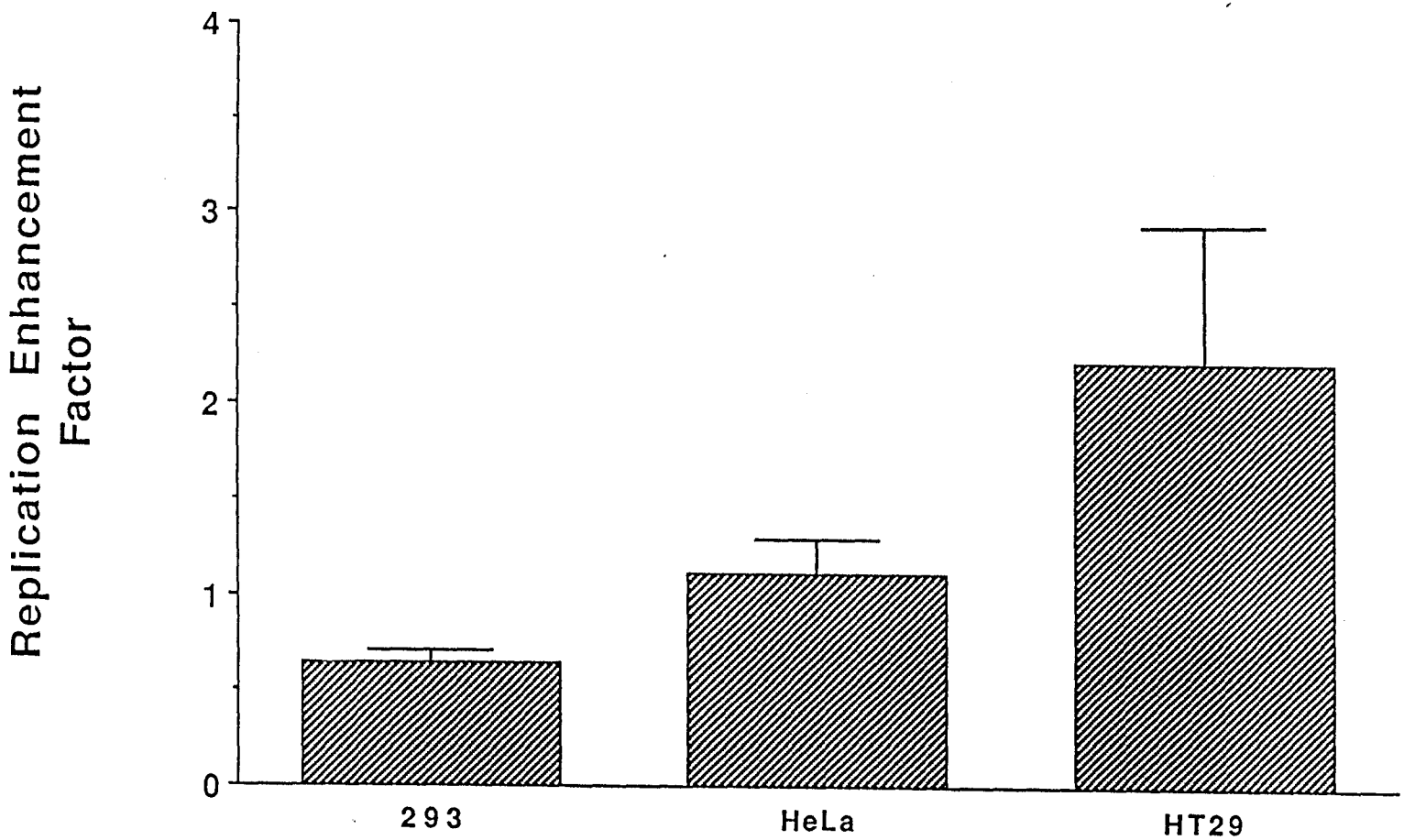

Replication Enhancement Factor
4
3
2
1
0
GM969
XP1BR

Figure 45: Histogram of viral DNA replication enhancement following infection of 293, HeLa, and HT29 cells with Ad5(ERCC1)

In individual experiments, which included 293, HeLa, and/or HT29 cells, infection with unirradiated Ad5(ERCC1) or Ad5(LacZ) was carried out at a MOI of 40 PFU/cell and viral DNA synthesis was scored at 36 hours p.i. A replication enhancement factor (REF), a ratio describing the amount of Ad5(ERCC1) DNA synthesized compared to the amount of Ad5(LacZ) DNA synthesized, was calculated for each cell line in each experiment. The mean Ad5(ERCC1) REF of each cell line for a number of experiments, as indicated in Table 16, was calculated and is presented in this figure. Error bars are standard error.


Replication Enhancement Factor
4
3
2
1
0
293
HeLa
HT29

in HeLa, CHO, and mouse L cells which are permissive, semi-permissive, and non-permissive, respectively, for Ad infection.

D.1. Replication of Ad5(LacZ) DNA in Human, Hamster, and Murine Cells

HeLa, AA8, and L cells were infected with Ad5(LacZ) at a MOI of 40 PFU/cell. The viral DNA was extracted from the cells at various time points p.i. and quantified by slot-blotting, hybridization to a radioactive Ad DNA probe, and scintillation counting. The results of this experiment are presented in Figure 46. HeLa cells are the first to begin synthesis of viral DNA, followed by AA8. HeLa cells synthesize the most viral DNA while the amounts produced in AA8 cells are reduced approximately 2-fold compared to HeLa. L cells were found to replicate very little viral DNA. Within 36 hours, viral DNA synthesis in HeLa cells has reached its maximum, while viral DNA synthesis in AA8 cells is delayed with its maximum reached near 72 hours p.i.

D.2. Replication of Ad5(denV) DNA in Human, Hamster, and Murine Cells

Since *denV* was seen to affect viral DNA synthesis in a number of cell types when scored at a fixed time point, it was decided to examine the effect of denV on the time course of viral DNA in HeLa, AA8, and L cells.

Cells were infected with Ad5(denV) at a MOI of 40 PFU/cell. The viral DNA was extracted from the cells at various time points p.i. and quantified by slot-blotting, hybridization to a radioactive Ad DNA probe, and scintillation counting. The results of this experiment are presented in Figure 47.

Figure 46: Time course of viral DNA replication in human, hamster, and murine cells following infection with Ad5(LacZ)

The time course of viral DNA replication following infection of Ad5(LacZ) was investigated in human (HeLa), hamster (AA8), and murine (L) cells. Cells were infected at 40 PFU/cell and the viral DNA produced was isolated and extracted at the indicated time points.

Closed squares	- HeLa, Ad5(LacZ)
Closed circles	- AA8, Ad5(LacZ)
Closed triangles	- L cells, Ad5(LacZ)

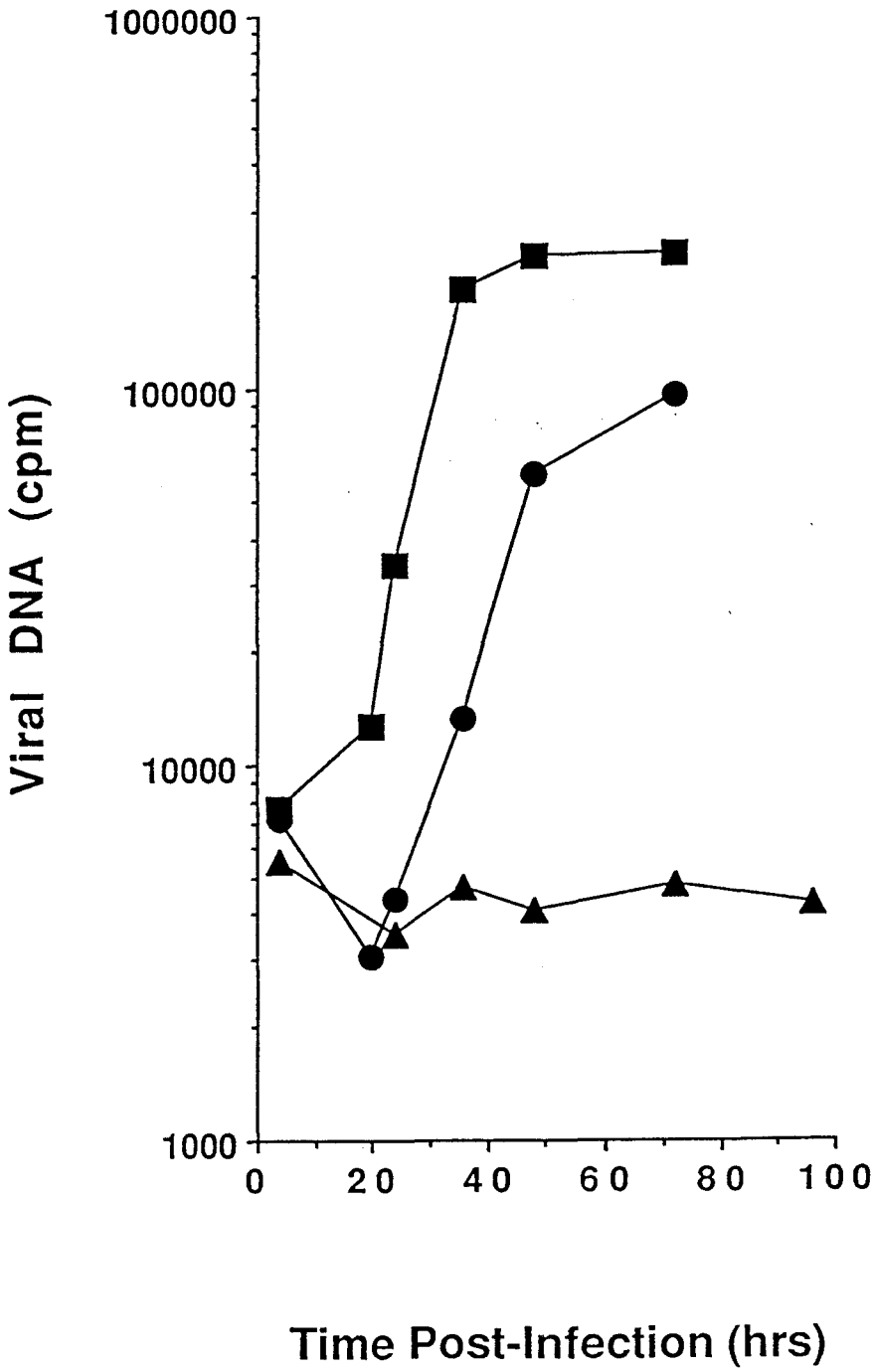
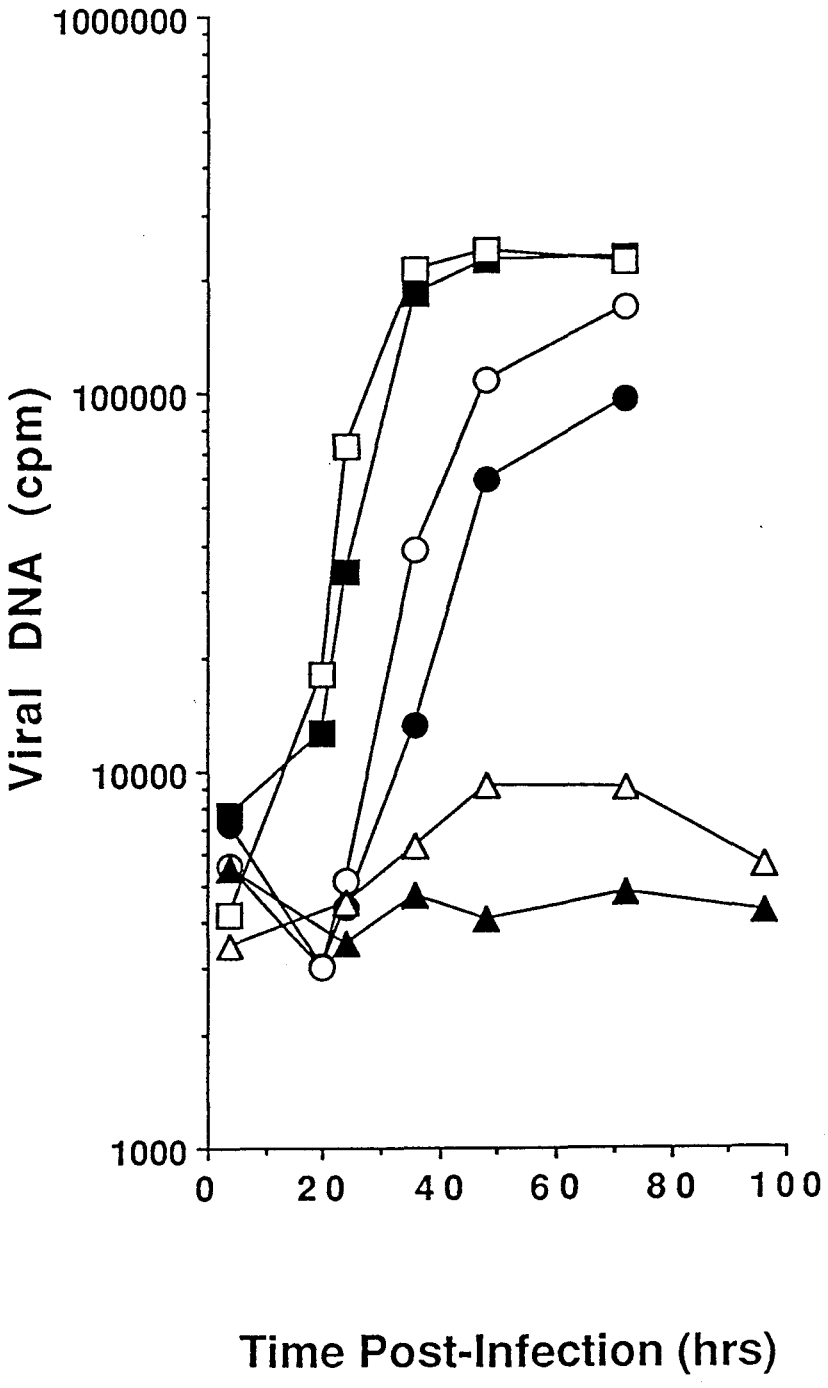

1000000
100000
10000
1000
Viral DNA (cpm)
0
20
40
60
80
100
Time Post-Infection (hrs)

Figure 47: Time course of viral DNA replication in human, hamster, and murine cells following infection with Ad5(denV)

The time course of viral DNA replication following infection of Ad5(denV) was investigated in human (HeLa), hamster (AA8), and murine (L) cells. Cells were infected at 40 PFU/cell and the viral DNA produced was isolated and extracted at the indicated time points. The time courses for DNA replication of Ad5(LacZ) in each cell type are included for comparison.

Closed squares	- HeLa, Ad5(LacZ)
Open squares	- HeLa, Ad5(denV)
Closed circles	- AA8, Ad5(LacZ)
Open circles	- AA8, Ad5(denV)
Closed triangles	- L cells, Ad5(LacZ)
Open triangles	- L cells, Ad5(denV)


1000000
100000
10000
1000
Viral DNA (cpm)
0
20
40
60
80
100
Time Post-Infection (hrs)

Replication of Ad5(denV) DNA in HeLa cells follows very similar kinetics to that of Ad5(LacZ) infection of HeLa cells. The rate of synthesis and the final amount synthesized are similar (Figure 47). Ad5(denV) DNA replication in AA8 cells is seen to be accelerated slightly compared to Ad5(LacZ) in AA8 although it does not proceed as quickly as the infection of the permissively infected HeLa cells. The amount of viral DNA synthesized in Ad5(denV) infected AA8 cells is also increased slightly compared to Ad5(LacZ) (Figure 47). L cells infected with Ad5(denV) replicate viral DNA somewhat more efficiently than Ad5(LacZ) infected L cells.

D.3. Replication of Ad5(ERCC1) in Human, Hamster, and Murine Cells

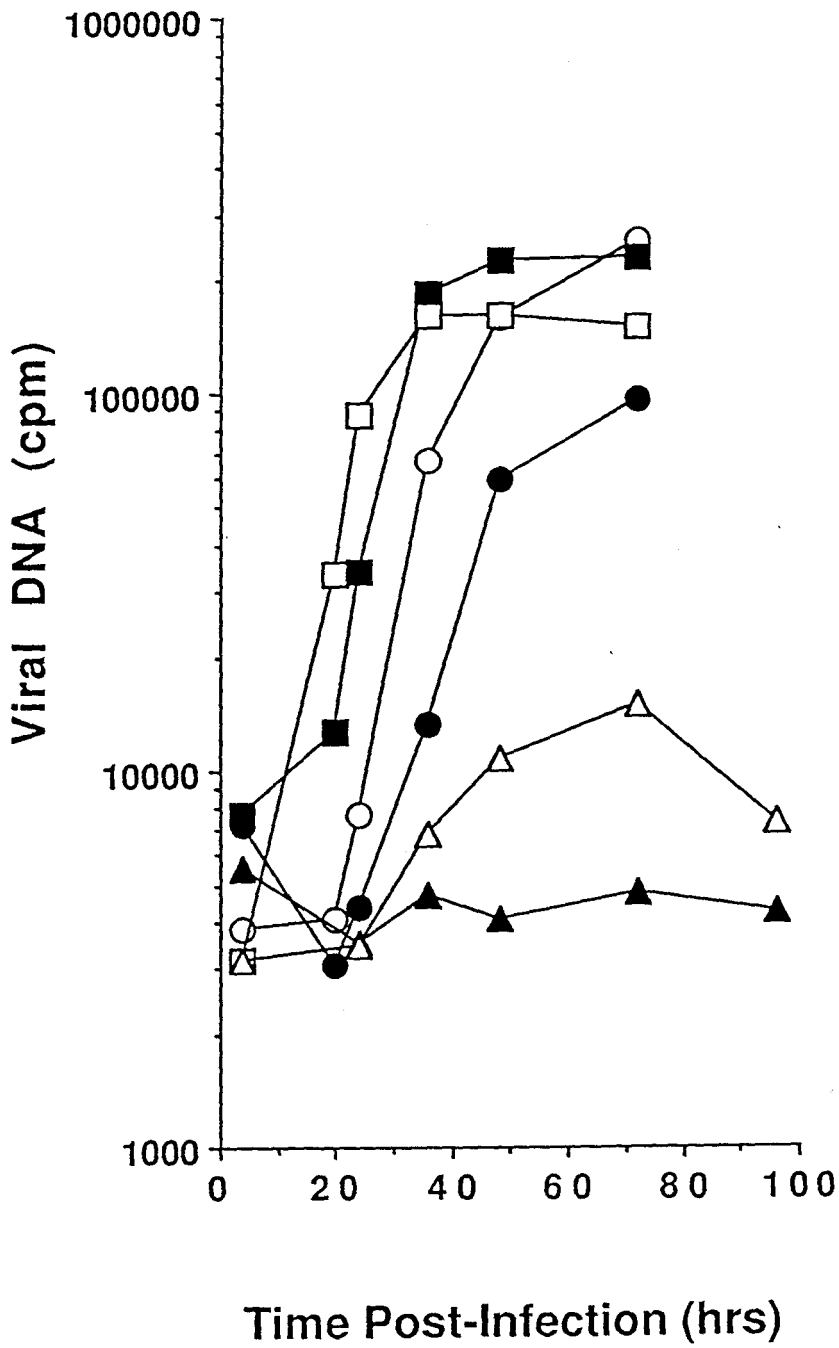
Given the possibility that, in addition to DNA repair, ERCC1 plays a role in DNA replication, it was considered important to examine the effect of ERCC1 on the time course of viral DNA synthesis in HeLa, AA8, and L cells.

The replication of Ad5(ERCC1) DNA in HeLa cells follows similar kinetics as that of Ad5(LacZ) or Ad5(denV) infection of HeLa cells with equivalent amounts of viral DNA being synthesized (Figure 48). The viral DNA replication kinetics of Ad5(ERCC1) infected AA8 cells are accelerated compared to Ad5(LacZ) infected AA8 cells (Figure 48). The amount of viral DNA synthesized by 72 hours p.i. in Ad5(ERCC1) infected AA8 cells is approximately two-fold the level synthesized in Ad5(LacZ) infected AA8 cells at the same time. Infection of L cells with Ad5(ERCC1) results in increased amounts of viral DNA being synthesized compared to Ad5(LacZ) infected L cells although the increase does not approach the levels synthesized in HeLa or AA8 cells (Figure 48).

Figure 48: Time course of viral DNA replication in human, hamster, and murine cells following infection with Ad5(ERCC1)

The time course of viral DNA replication following infection of Ad5(ERCC1) was investigated in human (HeLa), hamster (AA8), and murine (L) cells. Cells were infected at 40 PFU/cell and the viral DNA produced was isolated and extracted at the indicated time points. The time courses for DNA replication of Ad5(LacZ) in each cell type are included for comparison.

Closed squares	- HeLa, Ad5(LacZ)
Open squares	- HeLa, Ad5(ERCC1)
Closed circles	- AA8, Ad5(LacZ)
Open circles	- AA8, Ad5(ERCC1)
Closed triangles	- L cells, Ad5(LacZ)
Open triangles	- L cells, Ad5(ERCC1)


1000000
100000
10000
1000
Viral DNA (cpm)
0
20
40
60
80
100
Time Post-Infection (hrs)

E. Adenoviral Protein Synthesis in Mammalian Cells

As the kinetics of Ad5(denV) and Ad5(ERCC1) DNA replication were found to be altered compared to that of Ad5(LacZ) in AA8 cells and in L cells, it was considered of interest to investigate the production of viral proteins following infection of HeLa, AA8, and L cells with the three recombinant viruses. Late structural proteins, E2-encoded Ad DNA binding protein (Ad DBP), and E1A were the viral proteins which were chosen to be examined.

E.1. Production of Viral Structural Proteins in Human, Hamster, and Murine Cells infected with Ad5(LacZ)

HeLa, AA8, and L cells were seeded in 60 mm dishes and infected with Ad5(LacZ) at a MOI of 40 PFU/cell. At various time points p.i., the cells were labeled with ^{35}S-methionine after which the proteins were collected, immunoprecipitated with a rabbit polyclonal antisera made against Ad virions (164Rainbow and Howes 1982) and the immunoprecipitates run out on a SDS-polyacrylamide gel. Subsequent to drying of the gel, an autoradiograph was produced by exposing the gel to X-ray film for a period of 9 hours.

Three of the more plentiful viral late structural proteins produced during a permissive adenovirus infection are hexon, penton base, and fibre. Hexon (108 kDa), pentonbase (85 kDa), and fibre (62 kDa) are denoted by the first, second, and fourth arrows in Figure 49. This experiment revealed large amounts of hexon, pentonbase, and fibre being produced in the HeLa cells at 24, 51, and 72 hours p.i. The third arrow identifies the band representing Ad DNA binding

Figure 49: Production of viral structural antigens in human, hamster, and murine cells following infection with Ad5(LacZ)

60 mm plates of cells were infected with Ad5(LacZ) at 40 PFU per cell and labelled with ^{35}S-methionine for a 2 hour period at the specified times. The protein was harvested and one quarter of each sample was immunoprecipitated with a rabbit polyclonal antisera made against Ad virions. The immunoprecipitate was then run out on an SDS-polyacrylamide gel. After drying, the gel was placed on X-ray film to produce an autoradiograph. This figure represents autoradiographic exposure of 9 hours. The four bands denoted by the arrows are, from top to bottom, hexon (108 kDa), penton base (85 kDa), Ad DNA binding protein (72 kDa), and fibre (62 kDa).
Molecular weight standards (kDa) are indicated on the left of the figure.

116.25
97.4
66.2
45
A. HeLa
B. AA8
C. L cells
Mock
12 hrs
24 hrs
51 hrs
72 hrs
Mock
12 hrs
24 hrs
51 hrs
72 hrs
Mock
12 hrs
24 hrs
51 hrs
72 hrs

protein (Ad DBP) (72 kDa) which is also recognized by the antisera used. It can be seen that the rate of production of Ad DBP is highest at 12 hours post-infection in HeLa cells and decreases after this time.

Figure 49 also presents the same time course of viral structural protein synthesis in AA8 cells infected with Ad5(LacZ). The absolute amount of hexon, pentonbase, and fibre produced in the infection of AA8 cells is reduced considerably compared to Ad5(LacZ) infection of HeLa cells. The amount of hexon and fibre produced relative to Ad DBP is also decreased compared to a HeLa infection with Ad5(LacZ). Ad DBP is only detectable at 24 hours p.i. in AA8 cells infected with Ad5(LacZ) compared to its presence at 12 hours p.i. in HeLa cells.

The only protein detectable in the L cell infection with Ad5(LacZ) is the Ad DBP which is seen in small amounts at 51 and 72 hours p.i. (Figure 49).

E.2. Production of Viral Structural Proteins in Human, Hamster, and Murine Cells infected with Ad5(denV)

HeLa, AA8, and L cells were seeded in 60 mm dishes and infected with Ad5(LacZ) at a MOI of 40 PFU/cell. At various time points p.i., the cells were labeled with ^{35}S-methionine after which the proteins were collected, immunoprecipitated with a rabbit polyclonal antisera made against Ad virions (Rainbow and Howes 1982) and the immunoprecipitates run out on a SDS-polyacrylamide gel. Subsequent to drying of the gel, an autoradiograph was produced by exposing the gel to X-ray film for a period of 9 hours.

A comparison of Figures 49 and 50 reveals that the production of late viral structural proteins occurs earlier in HeLa cells infected with Ad5(denV)than in HeLa cells infected with Ad5(LacZ). In the Ad5(denV) infection, hexon is definitely visible at 12 hours p.i. whereas in the Ad5(LacZ) infection, it was not detectable at this time (Figure 49). Pentonbase and fibre are being produced in small amounts at 12 hours p.i. following Ad5(denV) infection but were not detectable at this time after the Ad5(LacZ) infection (Figure 49).

The production of viral structural proteins in AA8 cells infected with Ad5(denV) occurred earlier than in AA8 cells infected with Ad5(LacZ) (Figures 49 and 50). Ad DBP is being synthesized at 12 hours p.i. in the Ad5(denV) infected AA8 cells (Figure 50) but it is not seen at this time in the Ad5(LacZ) infected AA8 cells (Figure 49). Production of pentonbase is first detectable at 24 hours p.i. in both Ad5(LacZ) and Ad5(denV) infected AA8 cells, although greater amounts of this protein are being produced at this time in the Ad5(denV) infected cells. The synthesis of fibre is seen at 24 hours p.i. in the Ad5(denV) infected AA8 cells but this is not detectable until 51 hours after the Ad5(LacZ) infection of AA8 cells.

The synthesis of Ad DBP is detectable at 24 hours p.i. in L cells while in the Ad5(LacZ) infected L cells this is first seen at 51 hours p.i. (Figure 50). There is also a tiny amount of hexon detectable in Ad5(denV) infected L cells whereas none was detectable in the Ad5(LacZ) infected L cells.

Figure 50: Production of viral structural antigens in human, hamster, and murine cells following infection with Ad5(denV)

60 mm plates of cells were infected with Ad5(denV) at 40 PFU per cell and labelled with ^{35}S-methionine for a 2 hour period at the specified times. The protein was harvested and one quarter of each sample was immunoprecipitated with a rabbit polyclonal antisera made against Ad virions. The immunoprecipitate was then run out on an SDS-polyacrylamide gel. After drying, the gel was placed on X-ray film to produce an autoradiograph. This figure represents autoradiographic exposure of 9 hours. The four bands denoted by the arrows are, from top to bottom, hexon (108 kDa), penton base (85 kDa), Ad DNA binding protein (72 kDa), and fibre (62 kDa).
Molecular weight standards (kDa) are indicated on the left of the figure.

116 25
97.4
66 2
45
A. HeLa
Mock
12 hrs
24 hrs
51 hrs
72 hrs
B. AA8
Mock
12 hrs
24 hrs
51 hrs
72 hrs
C. L cells
Mock
12 hrs
24 hrs
51 hrs
72 hrs

E.3. Production of Viral Structural Proteins in Human, Hamster, and Murine Cells infected with Ad5(ERCC1)

HeLa, AA8, and L cells were seeded in 60 mm dishes and infected with Ad5(LacZ) at a MOI of 40 PFU/cell. At various time points p.i., the cells were labeled with ^{35}S-methionine after which the proteins were collected, immunoprecipitated with a rabbit polyclonal antisera made against Ad virions (Rainbow and Howes 1982) and the immunoprecipitates run out on a SDS-polyacrylamide gel. Subsequent to drying of the gel, an autoradiograph was produced by exposing the gel to X-ray film for a period of 9 hours.

The Ad5(ERCC1) infection of HeLa cells was similar to that for the Ad5(LacZ) infection at 12 and 24 hours p.i. (Figure 51) Interestingly, the production of viral structural proteins in the Ad5(ERCC1) infected HeLa cells appears to shut down at some point between 24 and 51 hours p.i.

AA8 cells infected with Ad5(ERCC1) synthesize viral structural proteins at similar rates and with an approximately similar time course to Ad5(denV) infected AA8 cells (Figure 51, Figure 50). The rates of synthesis of hexon and fibre are slow relative to the HeLa infections. The Ad DBP is very visible at 12 hours p.i. in the Ad5(ERCC1) infected AA8 cells, earlier than the first indication of Ad DBP at 24 hours p.i. in Ad5(LacZ) infected AA8 cells.

The L cell infection with Ad5(ERCC1) results in earlier detectable synthesis of Ad DBP (Figure 51). The first indication of Ad DBP synthesis in the Ad5(ERCC1) infection of L cells is at 24 hours p.i. compared to 51 hours p.i. in the Ad5(LacZ) infected L cells (Figure 49). There is also a small amount of

Figure 51: Production of viral structural antigens in human, hamster, and murine cells following infection with Ad5(ERCC1)

60 mm plates of cells were infected with Ad5(ERCC1) at 40 PFU per cell and labelled with ^{35}S-methionine for a 2 hour period at the specified times. The protein was harvested and one quarter of each sample was immunoprecipitated with a rabbit polyclonal antisera made against Ad virions. The immunoprecipitate was then run out on an SDS-polyacrylamide gel. After drying, the gel was placed on X-ray film to produce an autoradiograph. This figure represents autoradiographic exposure of 9 hours. The four bands denoted by the arrows are, from top to bottom, hexon (108 kDa), penton base (85 kDa), Ad DNA binding protein (72 kDa), and fibre (62 kDa).
Molecular weight standards (kDa) are indicated on the left of the figure.

116.25
97.4
66.2
45
A. HeLa
Mock
12 hrs
24 hrs
51 hrs
72 hrs
B. AA8
Mock
12 hrs
24 hrs
51 hrs
72 hrs
C. L cells
Mock
12 hrs
24 hrs
51 hrs
72 hrs

hexon being made in the Ad5(ERCC1) infected L cells whereas this is not seen in the Ad5(LacZ) infected L cells.

E.4. Production of E2 - Encoded Ad DNA Binding Protein in Human, Hamster, and Murine Cells Infected with Ad5(LacZ), Ad5(denV), and Ad5(ERCC1)

Immunoprecipitations carried out with the polyclonal antisera directed against late viral structural proteins appeared also to bind to the Ad DBP (Figures 49, 50, 51). Experiments using a monoclonal directed against Ad DBP were then performed to confirm the Ad DBP results that were seen in the immunoprecipitation reactions with the polyclonal late structural protein antisera.

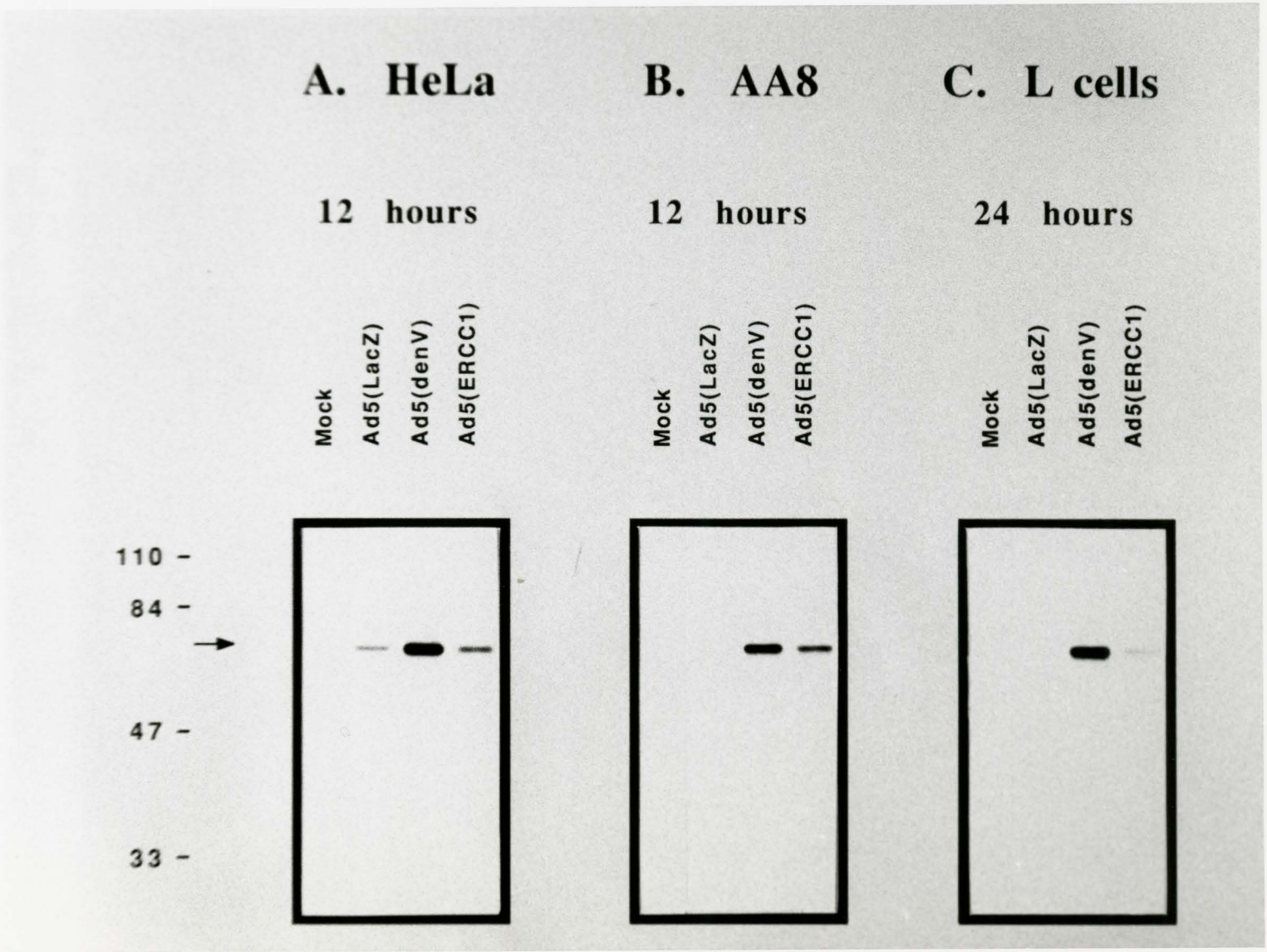
HeLa, AA8, and L cells were seeded in 60 mm dishes and infected with Ad5(LacZ), Ad5(denV), or Ad5(ERCC1) at a MOI of 40 PFU/cell. HeLa, AA8, and L cells were labeled with ^{35}S-methionine at 12 hours p.i., 12 hours p.i., and 24 hours p.i., respectively. The proteins were then collected, immunoprecipitated with H2-19 72K, a monoclonal antibody directed against Ad DBP (Rowe *et al.* 1984), and the immunoprecipitates run out on a SDS-polyacrylamide gel. Subsequent to drying of the gel, an autoradiograph was produced by exposing the gel to X-ray film for a period of 9 hours.

The rate of Ad DBP synthesis at 12 hours p.i. in HeLa cells was found to be highest for the Ad5(denV) infected cells with Ad5(ERCC1) and Ad5(LacZ) infected having somewhat lower rates of synthesis (Figure 52).

The Ad5(denV) infected AA8 cells are producing greater quantities of Ad

Figure 52: Production of adenoviral DNA binding protein in human, hamster, and murine cells following infection with Ad5(LacZ), Ad5(denV), or Ad5(ERCC1)

60 mm plates of cells were infected with Ad5(LacZ), Ad5(denV), or Ad5(ERCC1) at 40 PFU per cell and labelled with ^{35}S-methionine for a 2 hour period at the specified times. The protein was harvested and one quarter of each sample was immunoprecipitated with a monoclonal antibody for adenoviral DNA binding protein. The immunoprecipitate was then run out on an SDS-polyacrylamide gel. After drying, the gel was placed on X-ray film to produce an autoradiograph. This figure represents autoradiographic exposure of 9 hours. The band denoted by the arrow represents the adenoviral DNA binding protein. Molecular weight standards (kDa) are indicated on the left of the figure.


A. HeLa
B. AA8
C. L cells
12 hours
12 hours
24 hours
Mock
Ad5(LacZ)
Ad5(denV)
Ad5(ERCC1)
110 -
84 -
47 -
33 -

DBP at 12 hours p.i. than are the Ad5(ERCC1) infected AA8 cells (Figure 52). The Ad5(LacZ) infected cells are not producing detectable quantities of the Ad DBP at this time.

In the L cells, only Ad5(denV) infected cells are producing appreciable amounts of Ad DBP at 24 hours p.i. (Figure 52). The Ad5(ERCC1) infected cells are producing small amounts of Ad DBP while the L cells infected with Ad5(LacZ) do not produce detectable quantities of Ad DBP at 24 hours p.i.

E.5 Production of E1A Protein in Human, Hamster, and Murine Cells Infected with Ad5(LacZ), Ad5(denV), and Ad5(ERCC1)

HeLa, AA8, and L cells were seeded in 60 mm dishes and infected with Ad5(LacZ) at a MOI of 40 PFU/cell. HeLa, AA8, and L cells were labelled with ^{35}S-methionine at 12 hours p.i., 12 hours p.i., and 24 hours p.i., respectively. The proteins were then collected, immunoprecipitated with a monoclonal antibody directed against E1A (Harlow *et al.* 1985) and the immunoprecipitates run out on a SDS-polyacrylamide gel. Subsequent to drying of the gel, an autoradiograph was produced by exposing the gel to X-ray film for a period of 9 hours.

The impetus behind this investigation was to determine if the amount of E1A differed following infection with these three recombinant viruses. The time points used in this investigation were chosen as these were the times when Ad DBP was seen to appear in the Ad5(denV) and Ad5(ERCC1) infections but not in the Ad5(LacZ) infections.

The immunoprecipitations of Ad5(LacZ), Ad5(denV), and Ad5(ERCC1) infected HeLa cells with the E1A monoclonal antibody resulted in three bands in the vicinity of the 47 kDa marker (Figure 53). The amount of E1A appears greatest in the Ad5(denV) infected cells with intermediate amounts in the Ad5(LacZ) infected cells and the Ad5(ERCC1) infected cells with the least.

The AA8 cells infected with Ad5(LacZ), Ad5(denV), or Ad5(ERCC1) were found to produce lesser amounts of E1A at 12 hours p.i. compared to HeLa at this same time (Figure 53). The band representing the largest species of E1A is the only band visible in the AA8 infected cells. The amounts of this band appear very similar in these three infections.

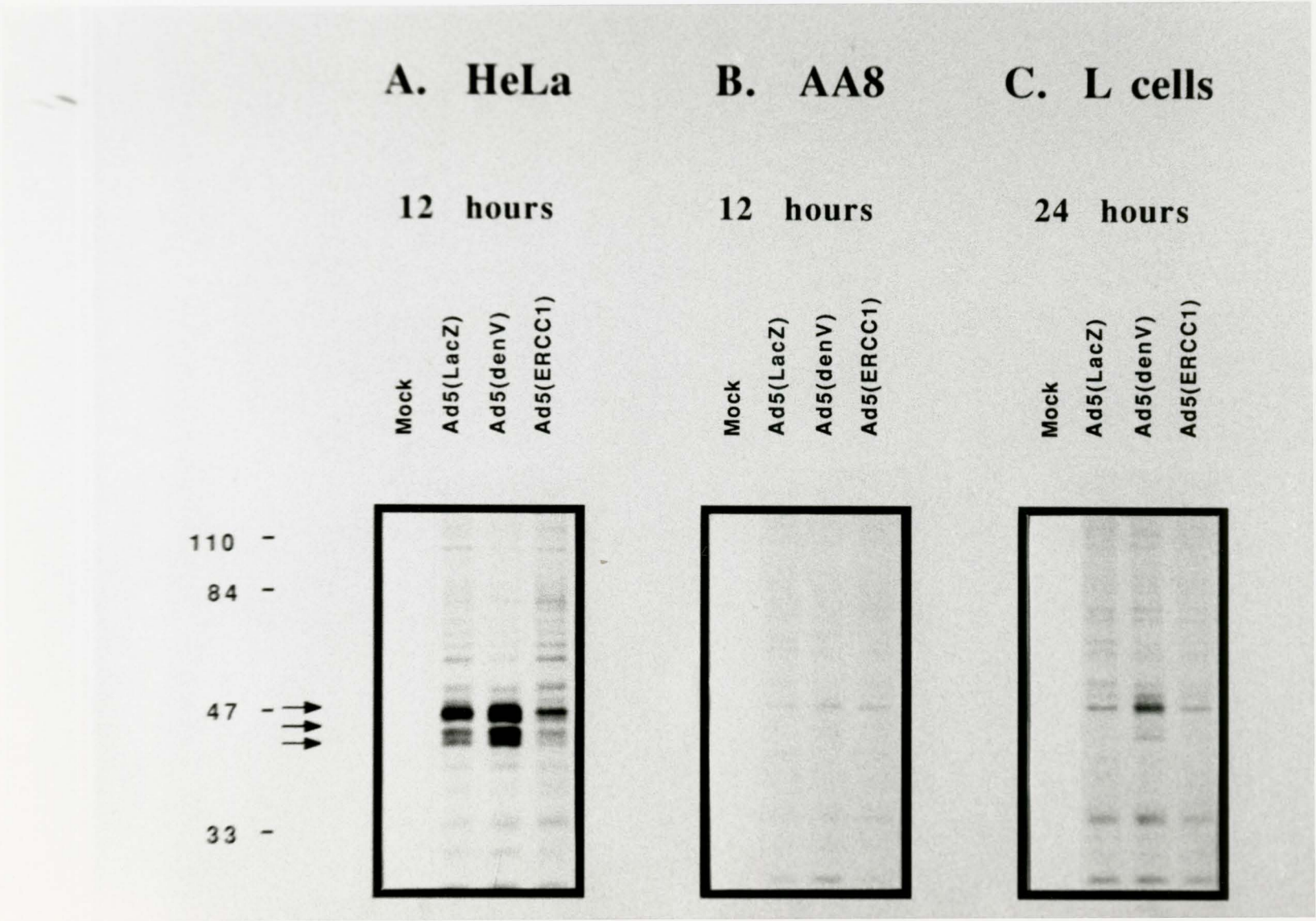
The Ad5(LacZ), Ad5(denV), and Ad5(ERCC1) infected L cells synthesized relatively little amounts of E1A at 24 hours p.i. The relative amounts of E1A produced in these cells infected with the different recombinant viruses were roughly equal with Ad5(denV) infected cell perhaps producing slightly greater quantities of E1A (Figure 53).

F. UV Enhanced Reactivation of UV-irradiated Adenovirus in CHO Cells

Enhanced reactivation (ER) of virus treated with a DNA damaging agent following infection of cells, pre-treated with DNA damaging agents, has been observed in many cell types (Rainbow 1981; Defais *et al.* 1983). Results of ER in rodent cells have been limited to date with no previous results of ER of Ad in CHO cells. This study investigated the ability of UV-irradiated CHO cells to reactivate viral DNA synthesis of UV-irradiated Ad 5.

Figure 53: Production of E1A protein in human, hamster, and murine cells following infection with Ad5(LacZ), Ad5(denV), or Ad5(ERCC1)

60 mm plates of cells were infected with Ad5(LacZ), Ad5(denV), or Ad5(ERCC1) at 40 pfu per cell and labelled with ^{35}S-methionine for a 2 hour period at the specified times. The protein was harvested and one quarter of each sample was immunoprecipitated with a monoclonal antibody for E1A protein. The immunoprecipitate was then run out on an SDS-polyacrylamide gel. After drying, the gel was placed on X-ray film to produce an autoradiograph. This figure represents autoradiographic exposure of 7 days. The three bands denoted by the arrows represent differentially phosphorylated species of E1A. Molecular weight standards (kDa) are indicated on the left of the figure.


A. HeLa
B. AA8
C. L cells
12 hours
12 hours
24 hours
Mock
Ad5(LacZ)
Ad5(denV)
Ad5(ERCC1)
110
84
47
33

A typical time course of Ad 5 DNA synthesis in AA8 cells irradiated with various UV fluences immediately prior to infection with unirradiated Ad is depicted in Figure 54. A repeat experiment (not shown) was found to yield results which were essentially the same. UV fluences of 5 J/m^2 and 10 J/m^2 to the cells were found to enhance the production of viral DNA. Viral DNA synthesis in AA8 cells which were UV-irradiated with UV fluences of 5 J/m^2, 10 J/m^2, and 15 J/m^2 immediately prior to infection with UV-irradiated Ad was also increased compared to that in unirradiated cells (Figure 55).

The relative amount of viral DNA synthesized at 49.5 hours p.i. for UV-irradiated virus was expressed as a fraction of that for unirradiated virus and these values were then plotted as a function of UV fluence to the cells (Figure 56a). UV enhanced reactivation (UVER) factors, calculated as the ratio of survival of viral DNA synthesis for irradiated virus to unirradiated virus, normalized to that value in unirradiated cells, were determined and plotted as a function of UV fluence to the cells. Figure 56a demonstrates that a UVER of CHO cells infected immediately after UV-treatment of the cells can not be detected when viral DNA is quantified at 49.5 hours p.i. However, a small UVER is detected at 15 J/m^2 to the cells if the time of viral DNA collection is 74 hours after infection (Figure 56b).

The effect on UVER of delaying infection of the cells following UV to the cells was examined. It was not possible to investigate delays much greater than 12 hours as the cell sheet began to lift off the bottom of the wells after this time.

Viral DNA time courses are presented for infection of AA8 cells with unirradiated or UV-irradiated virus 12 hours after UV fluence to the cells in Figures 57 and 58, respectively. UV fluences of 5 J/m^2, 10 J/m^2, and 15 J/m^2 to

Figure 54: Time course of viral DNA replication in CHO cells UV-irradiated immediately prior to infection with unirradiated wild-type Ad5.

CHO cells were UV-irradiated with various fluences immediately prior to infection with 40 PFU cell of unirradiated wild-type Ad 5. This figure represents the subsequent time course of viral DNA replication.

Open circles	- 0 J/m2 to cells
Open squares	- 5 J/m2 to cells
Open triangles	- 10 J/m2 to cells
Crosses	- 15 J/m2 to cells

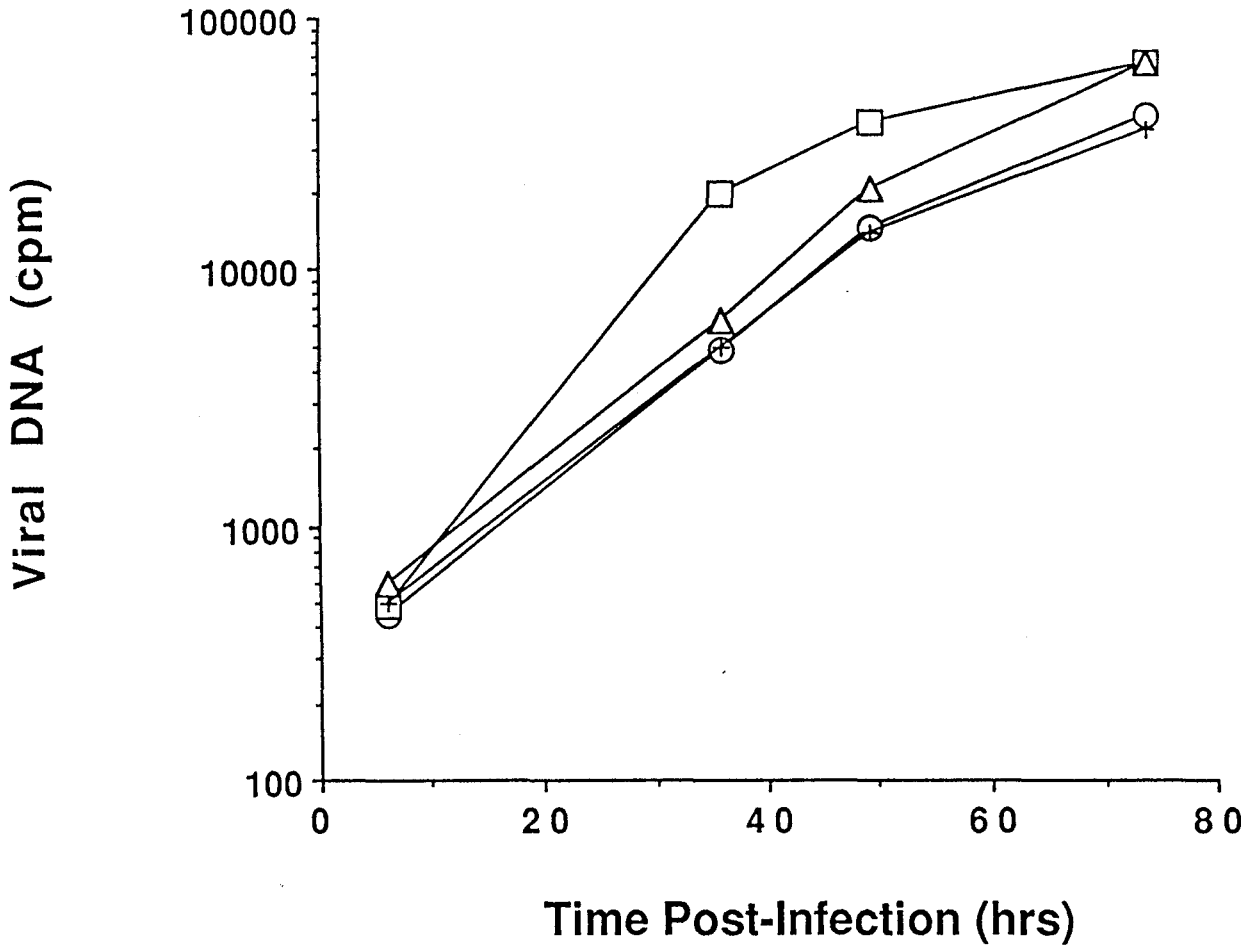

100000
10000
1000
100
0
20
40
60
80
Viral DNA (cpm)
Time Post-Infection (hrs)

Figure 55: Time course of viral DNA replication in CHO cells UV-irradiated immediately prior to infection with UV-irradiated wild-type Ad 5.

CHO cells were UV-irradiated with various fluences immediately prior to infection with 40 PFU per cell of wild-type Ad 5 which had been UV-irradiated with a fluence of 60 J/m^2. This figure represents the subsequent time course of viral DNA replication.

Closed circles	- 0 J/m2 to cells
Closed squares	- 5 J/m2 to cells
Closed triangles	- 10 J/m2 to cells
Crosses	- 15 J/m2 to cells

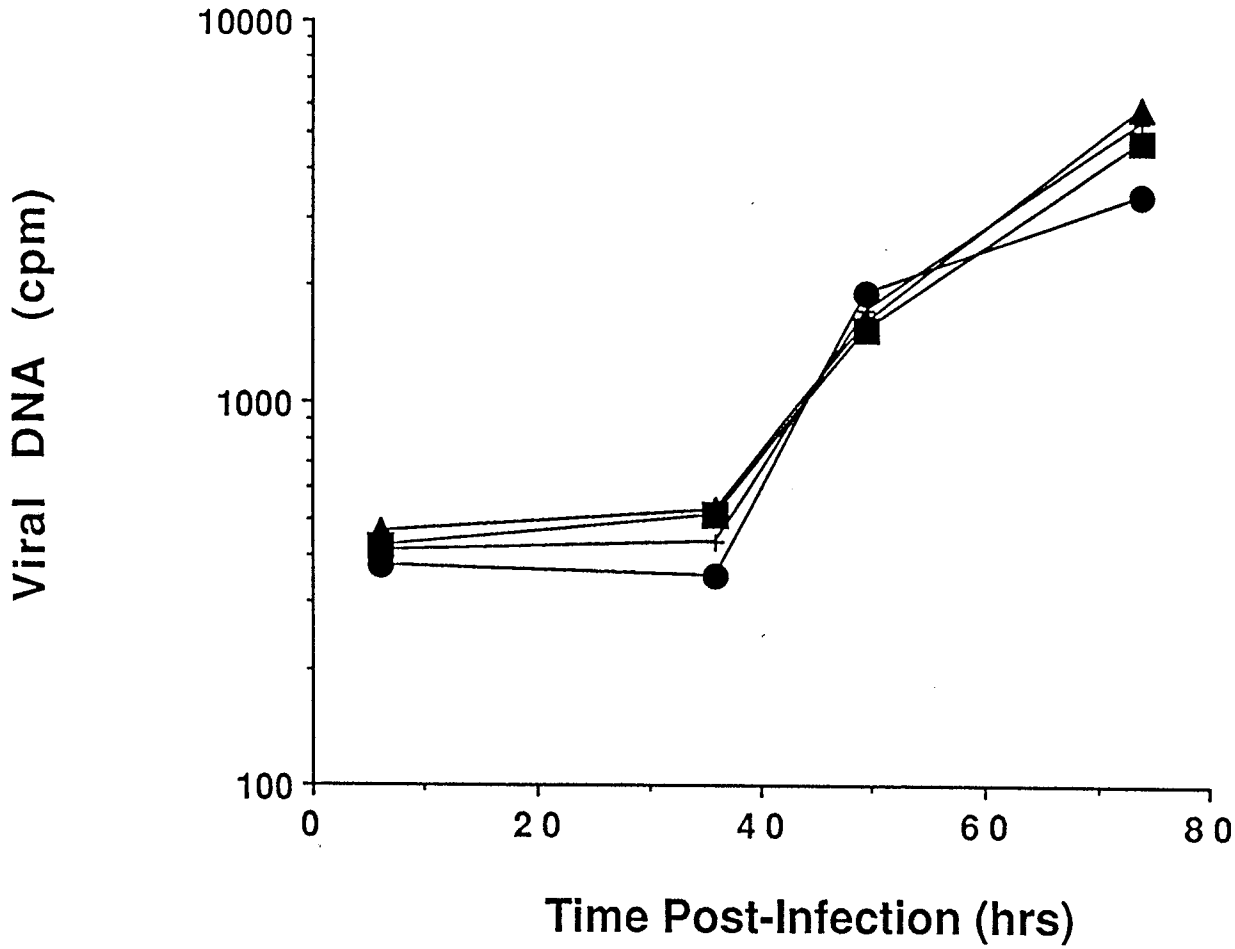

10000
1000
100
Viral DNA (cpm)
0
20
40
60
80
Time Post-Infection (hrs)

Figure 56a: UV-survival of viral DNA replication in CHO cells UV-irradiated immediately prior to infection with wild-type Ad 5.

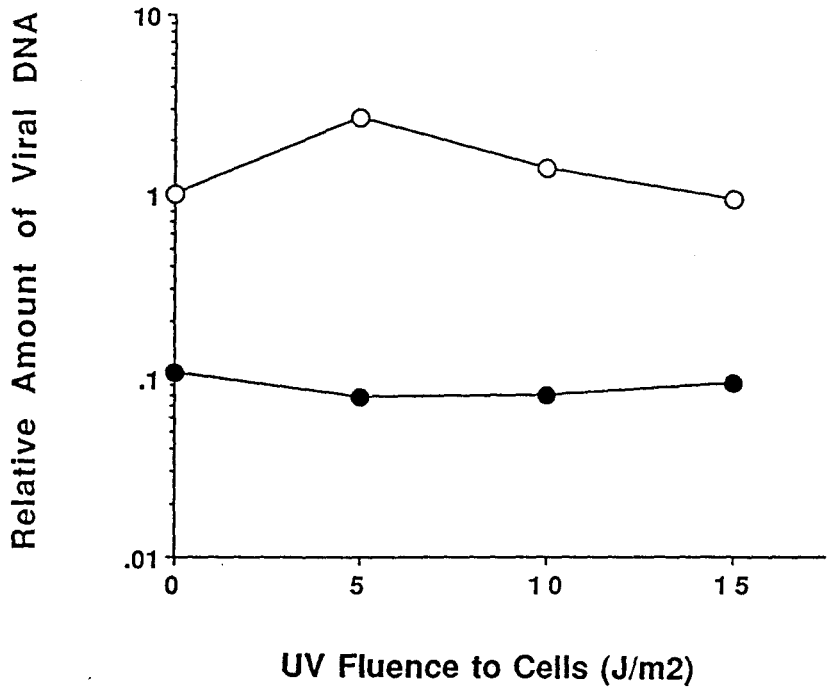
Upper panel:

The amount of viral DNA produced by 49.5 hours post-infection in CHO cells UV-irradiated immediately prior to infection was expressed as a fraction of that produced in unirradiated CHO cells infected with unirradiated virus. These data points were then plotted as a function of UV fluence to the cells.

Open circles	- 0 J/m2 to the virus
Closed circles	- 60 J/m2 to the virus

Lower panel:

The normalized ratio of the survival of viral DNA synthesis in cells infected with UV-irradiated virus to that in cells infected with unirradiated virus is termed the UV enhanced reactivation (UVER) factor. This figure expresses the UVER as a function UV fluence to the cells.


Relative Amount of Viral DNA
10
1
.1
.01
0
5
10
15
UV Fluence to Cells (J/m2)

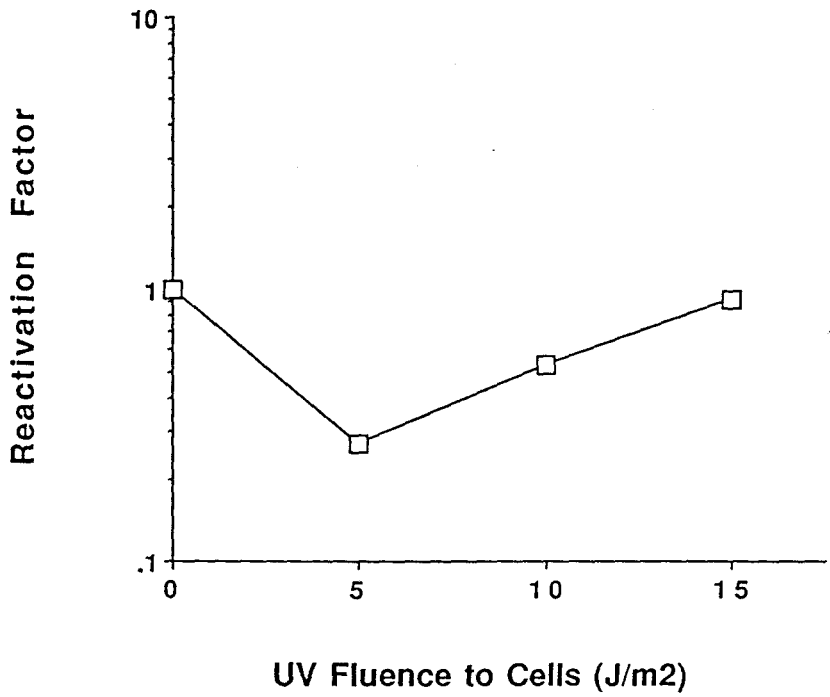

Reactivation Factor
10
1
.1
0
5
10
15
UV Fluence to Cells (J/m2)

Figure 56b: UV-survival of viral DNA replication in CHO cells UV-irradiated immediately prior to infection with wild-type Ad 5.

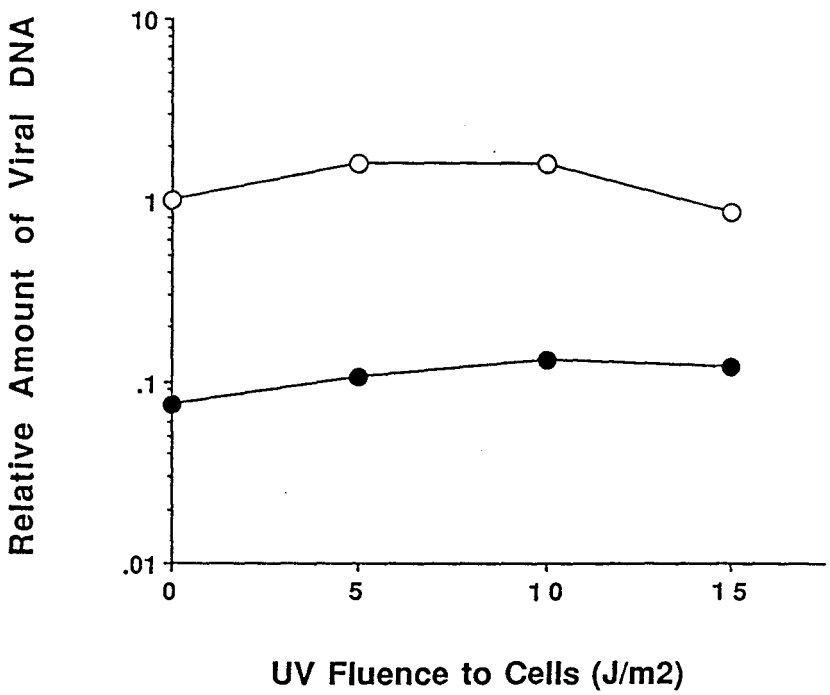
Upper panel:

The amount of viral DNA produced by 74 hours post-infection in CHO cells UV-irradiated immediately prior to infection was expressed as a fraction of that produced in unirradiated CHO cells infected with unirradiated virus. These data points were then plotted as a function of UV fluence to the cells.

Open circles	- 0 J/m2 to the virus
Closed circles	- 60 J/m2 to the virus

Lower panel:

The normalized ratio of the survival of viral DNA synthesis in cells infected with UV-irradiated virus to that in cells infected with unirradiated virus is termed the UV enhanced reactivation (UVER) factor. This figure expresses the UVER as a function UV fluence to the cells.


Relative Amount of Viral DNA
10
1
.1
.01
0
5
10
15
UV Fluence to Cells (J/m2)

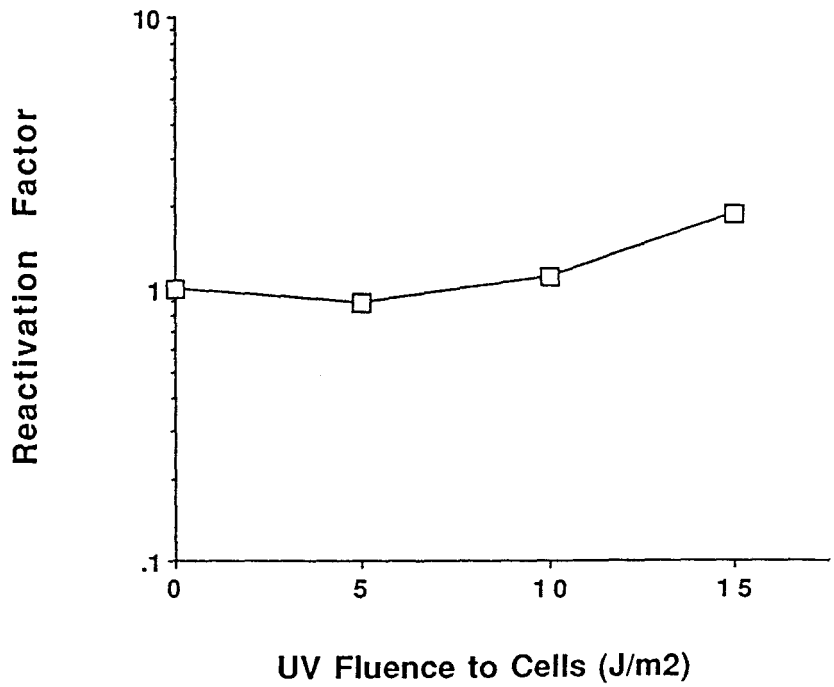

Reactivation Factor
10
1
.1
0
5
10
15
UV Fluence to Cells (J/m2)

Figure 57: Time course of viral DNA replication in CHO cells UV-irradiated 12 hours prior to infection with unirradiated wild-type Ad 5.

CHO cells were UV-irradiated with various fluences 12 hours prior to infection with 40 PFU per cell of unirradiated wild-type Ad 5. This figure represents the subsequent time course of viral DNA replication.

Open circles	- 0 J/m2 to cells
Open squares	- 5 J/m2 to cells
Open triangles	- 10 J/m2 to cells
Crosses	- 15 J/m2 to cells

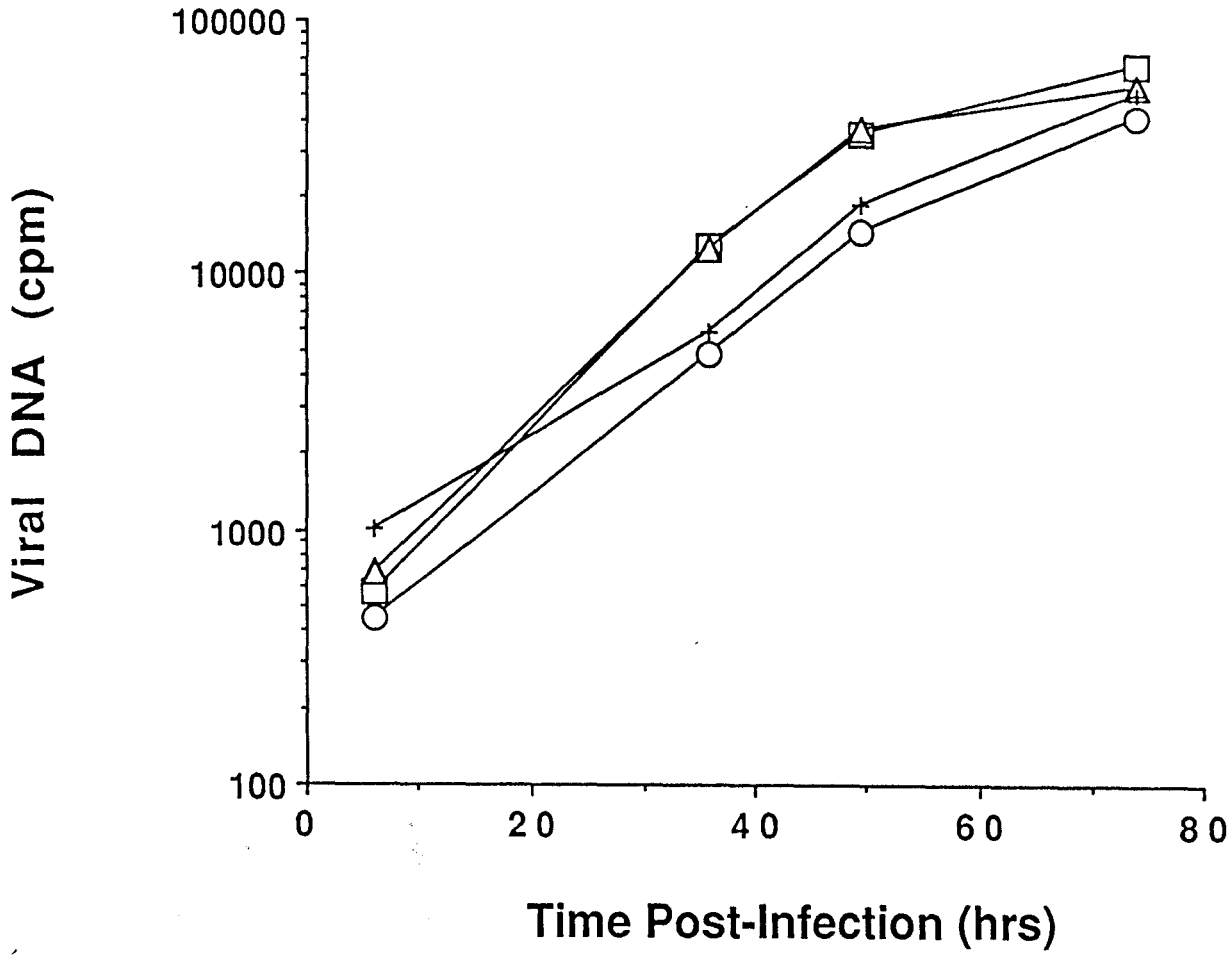

100000
10000
1000
100
0
20
40
60
80
Viral DNA (cpm)
Time Post-Infection (hrs)

Figure 58: Time course of viral DNA replication in CHO cells UV-irradiated 12 hours prior to infection with UV-irradiated wild-type Ad 5.

CHO cells were UV-irradiated with various fluences 12 hours prior to infection with 40 PFU per cell of wild-type Ad 5 which had been UV-irradiated with a fluence of 60 J/m^2. This figure represents the subsequent time course of viral DNA replication.

Closed circles	- 0 J/m2 to cells
Closed squares	- 5 J/m2 to cells
Closed triangles	- 10 J/m2 to cells
Crosses	- 15 J/m2 to cells

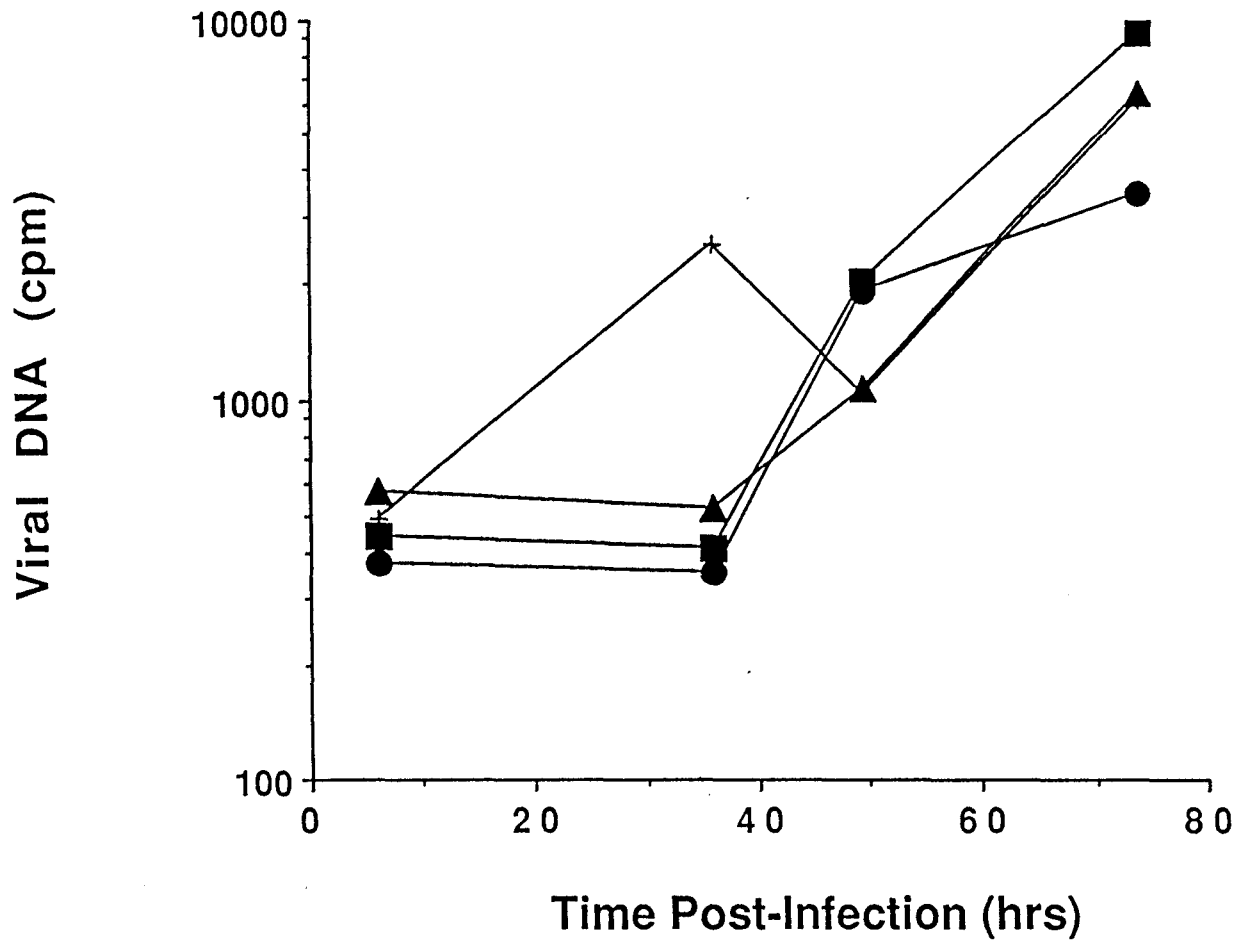

10000
1000
100
0
20
40
60
80
Viral DNA (cpm)
Time Post-Infection (hrs)

Figure 59a: UV-survival of viral DNA replication in CHO cells UV-irradiated 12 hours prior to infection with wild-type Ad 5.

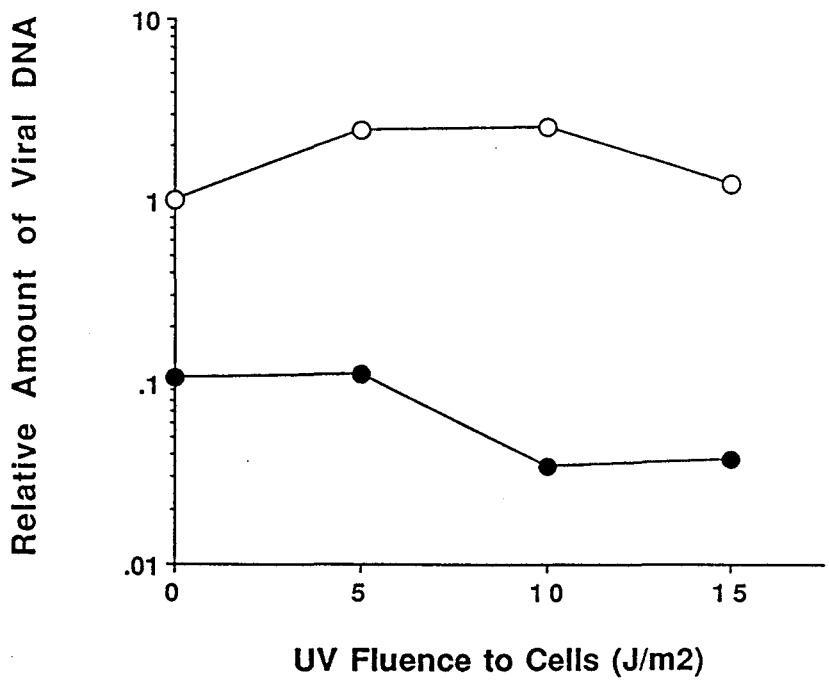
Upper panel:

The amount of viral DNA produced by 49.5 hours post-infection in CHO cells UV-irradiated 12 hours prior to infection was expressed as a fraction of that produced in unirradiated CHO cells infected with unirradiated virus. These data points were then plotted as a function of UV fluence to the cells.

Open circles	- 0 J/m2 to the virus
Closed circles	- 60 J/m2 to the virus

Lower panel:

The normalized ratio of the survival of viral DNA synthesis in cells infected with UV-irradiated virus to that in cells infected with unirradiated virus is termed the UV enhanced reactivation (UVER) factor. This figure expresses the UVER as a function UV fluence to the cells.


Relative Amount of Viral DNA
10
1
.1
.01
0
5
10
15
UV Fluence to Cells (J/m2)

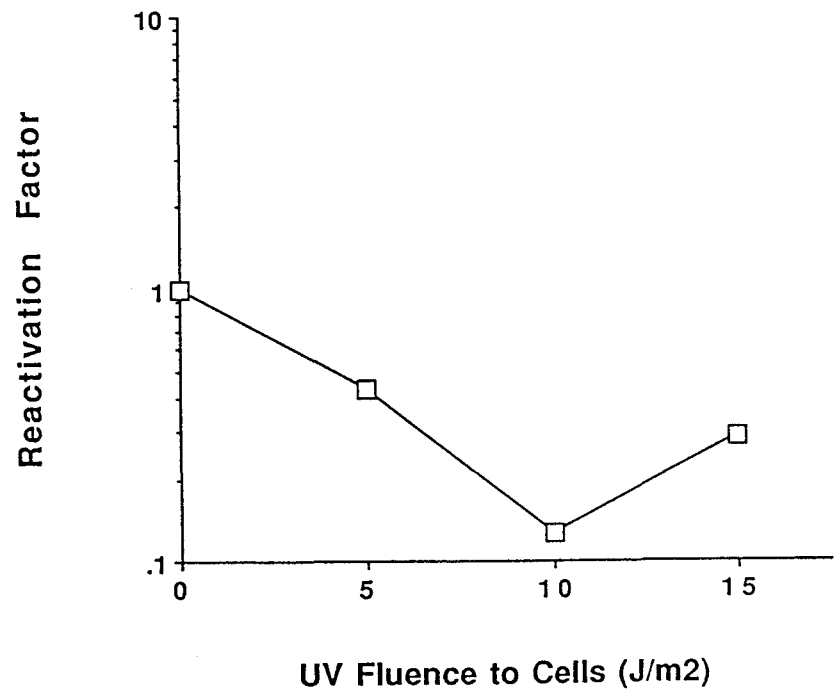

Reactivation Factor
10
1
.1
0
5
10
15
UV Fluence to Cells (J/m2)

Figure 59b: UV-survival of viral DNA replication in CHO cells UV-irradiated 12 hours prior to infection with wild-type Ad 5.

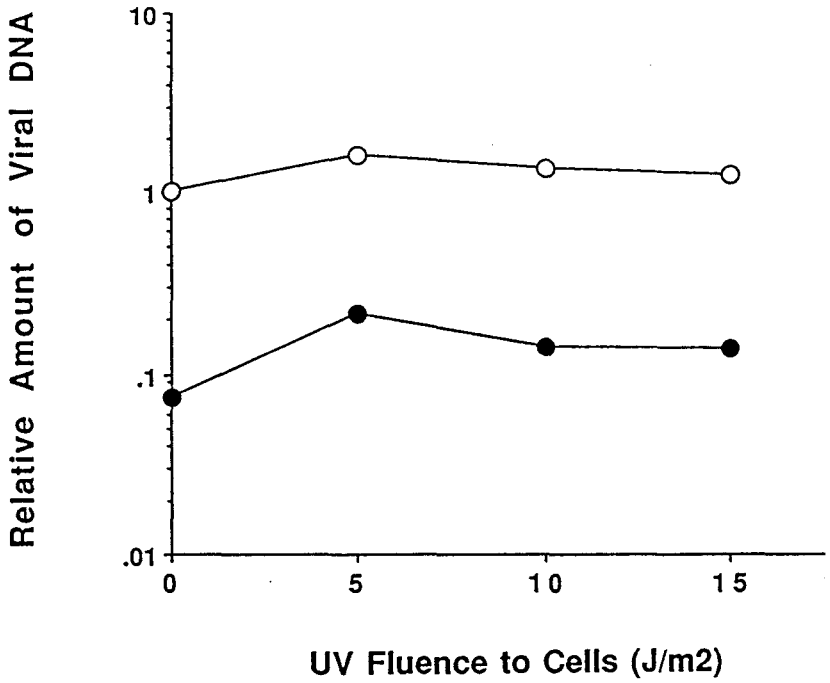
Upper panel:

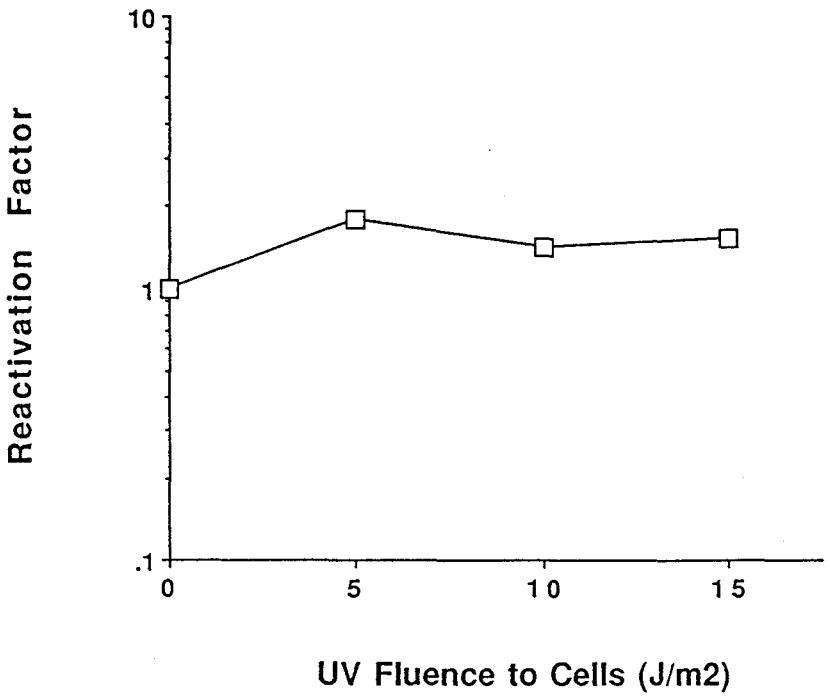
The amount of viral DNA produced by 74 hours post-infection in CHO cells UV-irradiated 12 hours prior to infection was expressed as a fraction of that produced in unirradiated CHO cells infected with unirradiated virus. These data points were then plotted as a function of UV fluence to the cells.

Open circles	- 0 J/m2 to the virus
Closed circles	- 60 J/m2 to the virus

Lower panel:

The normalized ratio of the survival of viral DNA synthesis in cells infected with UV-irradiated virus to that in cells infected with unirradiated virus is termed the UV enhanced reactivation (UVER) factor. This figure expresses the UVER as a function UV fluence to the cells.


Relative Amount of Viral DNA
10
1
.1
.01
0
5
10
15
UV Fluence to Cells (J/m2)


Reactivation Factor
10
1
.1
0
5
10
15
UV Fluence to Cells (J/m2)

the AA8 cells enhance viral DNA synthesis of both unirradiated and UV-irradiated virus. There is no UVER detectable for viral DNA synthesis when the amount of viral DNA is assessed at 49.5 hours p.i. (Figure 59a), although a small UVER can be detected at all UV exposures to the cells when viral DNA was examined at 74 hours p.i. (Figure 59b).

A small positive UVER value was detected in AA8 cells treated with a UV fluence of 15 J/m^2 immediately prior to infection of the cells if viral DNA was assessed at 74 hours p.i. Delaying infection of the cells after UV to the cells revealed positive UVER values for all UV fluences to the cells when viral DNA was scored at 74 hours p.i.

Tables

Table 1. Ad genome equivalent (Ad G.E.) to PFU ratio of various viral preparations.

Viral Prep	PFU/mL	Ad G.E./mL	Ad G.E./PFU
Ad5(ERCC1) - 1	2.8×10^9	1.06×10^{11}	37.8
Ad5(denV) - 1	2.0×10^9	1.59×10^{11}	79.5
Ad5(denV) - 2	5.0×10^9	1.16×10^{11}	23.2
Ad5(LacZ) - 1	2.3×10^9	3.27×10^{11}	142.4
Ad5(LacZ) - 2	1.8×10^9	2.62×10^{11}	145.0

Table 2. D_0 values for UV survival of Ad DNA synthesis obtained from least squares analysis of pooled points from all experiments for AA8 and its derived cells.

	Ad5(LacZ) (J/m^2)	Ad5(denV) (J/m^2)	Ad5(ERCC1) (J/m^2)	# of Expts
AA8	36.19 ± 4.09	152.97 ± 16.32	100.35 ± 10.97	17, 14, 14
UV20	8.19 ± 1.86	20.68 ± 2.49	33.41 ± 5.23	5, 4, 4
UV5	6.79 ± 0.98	24.13 ± 1.53	9.05 ± 1.36	6, 4, 4
UV24	8.04 ± 1.18	20.68 ± 2.77	8.04 ± 1.49	4, 3, 3
UV41	9.44 ± 1.66	27.14 ± 3.59	8.86 ± 2.17	5, 2, 2
UV135	11.14 ± 2.00	31.02 ± 2.01	12.77 ± 2.31	4, 3, 3
UV61	17.37 ± 3.33	109.15 ± 68.11	18.88 ± 6.40	3, 3, 3
5T4-12	21.71 ± 2.09	151.06 ± 12.65	102.50 ± 17.37	3, 3, 3
5-2ER2-6	61.40 ± 27.56	145.30 ± 26.21	148.48 ± 22.48	3, 3, 3
EM9	66.76 ± 10.45	215.10 ± 53.74	146.52 ± 37.59	4, 4, 4
UCL	8.52 ± 0.83	9.05 ± 0.47	43.43 ± 1.22	2, 2, 3

Table 3: %HCR of Ad5(LacZ) DNA synthesis following UV fluence to virus.

	% HCR	# of Expts
AA8 mutants		
UV20	22.6 ± 7.7	5
UV5	18.8 ± 4.8	6
UV24	22.2 ± 5.8	4
UV41	26.1 ± 7.5	5
UV135	30.8 ± 9.0	4
UV61	48.0 ± 14.6	3
5T4-12	60.0 ± 12.6	3
5-2ER2-6	169.7 ± 95.3	3
EM9	184.5 ± 49.7	4
UCL	23.5 ± 5.0	2
K1 mutants		
30PV	56.5 ± 36.2	4
50PV	104.9 ± 44.6	3
7PV	56.5 ± 46.9	3
4PV	141.6 ± 72.2	4
GM969 (normal)		
XP1BR group D	7.3 ± 2.5	2
293 (repair-proficient)		
HeLa	53.1 ± 7.7	4
HT29	17.3 ± 1.2	2

Table 4. D_0 values obtained for UV survival of Ad DNA synthesis from least squares analysis of pooled points from all experiments for K1 and its derived cells.

	Ad5(LacZ) (J/m^2)	Ad5(denV) (J/m^2)	Ad5(ERCC1) (J/m^2)	# of Expts
K1	51.23 ± 13.75	222.26 ± 42.20	211.34 ± 43.87	8, 8, 8
30PV	28.95 ± 10.77	227.38 ± 58.58	228.46 ± 35.70	4, 4, 4
50PV	53.74 ± 8.40	417.99 ± 117.40	272.11 ± 34.36	3, 3, 3
7PV	28.95 ± 16.28	140.96 ± 20.08	110.99 ± 12.22	3, 4, 4
4PV	72.52 ± 17.52	183.32 ± 54.35	121.86 ± 25.14	4, 6, 6

Table 5: Enhancement of UV survival of Ad DNA synthesis in AA8 and its derived mutants following Ad5(denV) or Ad5(ERCC1) infection.

	$\frac{D_0\ (denV)}{D_0\ (LacZ)}$	$\frac{D_0\ (ERCC1)}{D_0\ (LacZ)}$	# of Expts
Parental			
AA8	4.23 ± 0.93	2.77 ± 0.62	14,14
Mutants			
UV20	2.53 ± 0.88	4.08 ± 1.57	4,4
UV5	3.55 ± 0.74	1.33 ± 0.39	4,4
UV24	2.57 ± 0.72	1.00 ± 0.33	3,3
UV41	2.88 ± 0.87	0.94 ± 0.40	2,2
UV135	2.79 ± 0.68	1.15 ± 0.41	3,3
UV61	6.28 ± 5.13	1.09 ± 0.58	3,3
5T4-12	6.96 ± 1.25	4.72 ± 1.26	3,3
5-2ER2-6	2.37 ± 1.49	2.42 ± 1.45	3,3
EM9	3.22 ± 1.31	2.20 ± 0.91	4,4
UCL	1.06 ± 0.16	5.10 ± 0.64	2,3

Table 6: Enhancement of UV survival of Ad DNA synthesis in K1 and its derived mutants following Ad5(denV) or Ad5(ERCC1) infection.

	$\frac{D_0 \text{ (denV)}}{D_0 \text{ (LacZ)}}$	$\frac{D_0 \text{ (ERCC1)}}{D_0 \text{ (LacZ)}}$	# of Expts
Parental			
K1	4.34 ± 2.00	4.13 ± 1.96	8,8
Mutants			
30PV	7.85 ± 4.95	7.89 ± 4.17	4,4
50PV	7.78 ± 3.40	5.06 ± 1.43	3,3
7PV	4.87 ± 3.43	3.83 ± 2.58	4,4
4PV	2.53 ± 1.36	1.68 ± 0.75	6,6

Table 7. D_0 values for UV survival of Ad DNA synthesis obtained from least squares analysis of pooled points from all experiments for human cells.

	Ad5(LacZ) (J/m^2)	Ad5(denV) (J/m^2)	Ad5(ERCC1) (J/m^2)	# of Expts
FIBROBLASTS				
Normal				
GM969	100.14 ± 30.66	213.52 ± 37.58	215.00 ± 98.49	2
Mutant				
XP1BR group D	7.36 ± 0.26	18.10 ± 5.88	9.44 ± 1.08	2
TRANSFORMED CELLS				
293	179.53 ± 9.45	222.60 ± 24.68	219.78 ± 21.74	4
HeLa	95.30 ± 8.89	134.50 ± 6.48	147.12 ± 9.72	4
HT29	31.02 ± 0.55	50.65 ± 2.98	63.77 ± 5.75	2

Table 8: Enhancement of UV survival of Ad DNA synthesis in various human cell types following Ad5(denV) or Ad5(ERCC1) infection.

	$\frac{D_0\ (denV)}{D_0\ (LacZ)}$	$\frac{D_0\ (ERCC1)}{D_0\ (LacZ)}$	# of Expts
FIBROBLASTS			
Normal			
GM969	2.13 ± 1.03	2.15 ± 1.64	2,2
Mutant			
XP1BR group D	2.46 ± 0.89	1.28 ± 0.19	2,2
TRANSFORMED CELLS			
293	1.24 ± 0.20	1.22 ± 0.19	4,4
HeLa	1.41 ± 0.20	1.54 ± 0.25	4,4
HT29	1.63 ± 0.13	2.06 ± 0.22	2,2

Table 9. D_0 values for γ-ray survival of Ad DNA synthesis obtained from least squares analysis of pooled points from all experiments for AA8 and its derived cells.

	Ad5(LacZ) (krads)	Ad5(denV) (krads)	Ad5(ERCC1) (krads)	# of Expts
Parental				
AA8	437.27 ± 34.77	610.65 ± 110.75	600.60 ± 169.37	2
Mutants				
UV20	237.58 ± 66.37	307.79 ± 52.10	933.93 ± 288.16	2
UV41	223.98 ± 26.61	390.20 ± 54.36	571.67 ± 133.96	2
UV5	180.21 ± 35.92	335.10 ± 35.53	325.07 ± 88.63	1
EM9	335.10 ± 48.21	758.59 ± 285.64	885.01 ± 390.93	1

Table 10: Enhancement of γ-ray survival of Ad DNA synthesis in AA8 and some of its derived mutants following Ad5(denV) or Ad5(ERCC1) infection.

	$\frac{D_0 \text{ (denV)}}{D_0 \text{ (LacZ)}}$	$\frac{D_0 \text{ (ERCC1)}}{D_0 \text{ (LacZ)}}$	# of Expts
Parental			
AA8	1.40 ± 0.36	1.37 ± 0.50	2
Mutants			
UV20	1.30 ± 0.58	3.93 ± 2.31	2
UV41	1.74 ± 0.45	2.55 ± 0.90	2
UV5	1.86 ± 0.57	1.80 ± 0.85	1
EM9	2.26 ± 1.10	2.64 ± 1.55	1

Table 11. Replication of Ad5(LacZ) DNA in AA8 cells and its derived cell lines.

	Relative Viral DNA	# of Expts
AA8	100 ± 0	15
UV20	6.92 ± 2.75	3
UV5	90.37 ± 30.83	4
UV24	31.01 ± 15.52	3
UV41	248.00 ± 121.62	2
UV135	97.80 ± 14.69	3
UV61	71.31 ± 24.68	3
5T4-12	36.48 ± 17.52	3
5-2ER2-6	89.21 ± 37.04	3
EM9	77.75 ± 25.64	4
UCL	14.68 ± 4.91	2

Table 12. Replication of Ad5(LacZ) DNA in K1 cells and its derived cell lines.

	Relative Viral DNA	# of Expts
K1	100 ± 0	6
30PV	108.60 ± 29.80	4
50PV	118.90 ± 22.49	2
7PV	59.00 ± 23.00	4
4PV	90.30 ± 18.10	2

Table 13. Enhancement of viral DNA replication in AA8 cells and its derived cell lines following infection with recombinant Ad vectors carrying repair genes.

	REF - Ad5(denV)	REF - Ad5(ERCC1)	# of Expts
AA8	2.71 ± 0.62	3.15 ± 0.46	13
UV20	29.75 ± 12.57	19.88 ± 5.55	3
UV5	4.39 ± 1.94	5.40 ± 1.82	4
UV24	5.63 ± 2.75	8.46 ± 3.64	3
UV41	3.89 ± 0.77	3.09 ± 2.06	2
UV135	2.96 ± 1.50	2.73 ± 0.48	3
UV61	2.66 ± 1.03	3.38 ± 0.87	3
5T4-12	11.41 ± 2.52	7.55 ± 0.89	3
5-2ER2-6	3.81 ± 2.26	4.14 ± 0.83	3
EM9	4.89 ± 3.37	4.89 ± 2.93	4
UCL	3.58 ± 0.03	7.24 ± 2.23	2

Table 14. Enhancement of viral DNA replication in K1 cells and its derived cell lines following infection with recombinant Ad vectors carrying repair genes.

	REF - Ad5(denV)	REF - Ad5(ERCC1)	# of Expts
K1	6.45 ± 1.61	6.78 ± 1.49	5
30PV	3.59 ± 1.49	4.82 ± 2.53	4
50PV	4.02 ± 2.10	4.76 ± 2.69	3
7PV	27.72 ± 5.29	23.48 ± 7.29	3
4PV	3.17 ± 1.56	2.88 ± 0.94	4

Table 15. Replication of Ad5(LacZ) DNA in human cells.

	Relative Viral DNA	# of Expts
Fibroblasts		
GM969	100 ± 0	2
XP1BR group D	44.62 ± 4.18	2
Transformed Cells		
293	100 ± 0	4
HeLa	75.40 ± 24.79	4
HT29	20.70 ± 9.07	2

Table 16. Enhancement of viral DNA replication in human cells following infection with recombinant Ad vectors carrying repair genes.

	REF - Ad5(denV)	REF - Ad5(ERCC1)	# of Expts
Fibroblasts			
GM969	1.65 ± 0.02	1.37 ± 0.59	2
XP1BR group D	3.76 ± 1.37	2.94 ± 1.10	2
Transformed Cells			
293	0.88 ± 0.07	0.64 ± 0.06	4
HeLa	1.41 ± 0.19	1.12 ± 0.15	4
HT29	2.38 ± 1.05	2.22 ± 0.72	2

DISCUSSION

The main goal of this study was to combine the use of adenovirus (Ad) as a probe of cellular DNA repair and its potential as an expression vector for DNA repair genes to examine the repair deficiencies in Chinese hamster ovary cell mutants and the effects of exogenous DNA repair genes in these cells. Two recombinant Ad expression vectors encoding DNA repair genes were utilized in this study. Ad5(denV) has the bacteriophage T4 *denV* gene, under control of the Rous Sarcoma Virus promoter and SV40 polyadenylation sequences, inserted into the deleted E3 region of the Ad 5 genome (Colicos *et al.* 1991). The other recombinant Ad expression vector employed was Ad5(ERCC1) which contains the 1.1 kb cDNA of the human DNA repair gene, ERCC1, inserted into the deleted E3 region of the Ad 5 genome (Rainbow and Castillo 1992). These two Ad 5 recombinants along with Ad5(LacZ) control virus, which has the β-galactosidase gene inserted into the deleted E3 region of Ad 5, were used to infect cells and the UV survival of Ad DNA synthesis for the three viruses was examined.

As the study progressed, its scope was broadened to include an investigation of the UV survival of Ad DNA synthesis in human fibroblasts and human tumour cells following infection with the three Ad 5 recombinants. Additionally, an examination of the effects of the viral encoded *denV* and *ERCC1* gene products on the kinetics of viral DNA replication and viral protein

synthesis in permissive, semi-permissive, and non-permissive Ad infections was conducted.

Survival of Viral DNA Synthesis for UV-Irradiated Adenovirus in Mammalian Cells

This study used the endpoint of viral DNA synthesis to assess UV survival of virus in various cells. The most important advantage of the use of this endpoint is that it permits an examination of Ad survival in a range of cell types which, due to their semi-permissive nature, have not been assessed by other viral endpoints. In the past few years, CHO DNA repair mutants have been used very successfully in the isolation and cloning of a number of human DNA repair genes. Until now, there has been very little information gathered concerning the UV survival of virus in these cells as they are not permissive for Ad infection. Therefore, it is not possible to score for common viral endpoints such as plaque formation which result from a permissive infection. It has been observed, however, that CHO cells do replicate Ad DNA (Longiaru and Horwitz 1981; Eggerding and Pierce 1986) and this study has used this feature of CHO Ad infections as an endpoint to monitor UV survival of virus in these cells.

Viral DNA synthesis was assessed at 72 and 36 hours p.i. in CHO and human cells, respectively, as these times coincide with maximum viral DNA synthesis for non-irradiated virus in each cell type (Rainbow and Castillo 1992; this study). Initial experiments indicated that viral DNA synthesis of UV-irradiated virus was delayed compared to that of unirradiated virus (data not shown, Figure 54). This observation emphasized the importance of maintaining

consistent collection times of viral DNA among experiments as the DNA replication delay for the UV-irradiated virus would be emphasized to a greater degree at earlier harvest times. A smaller deficiency would be detected if collection of viral DNA was done at later times as the replication of UV-irradiated viral DNA would continue after that of non-irradiated virus had begun to plateau.

Viral DNA synthesis is an endpoint of virus survival that is influenced to a larger extent by variation in the time of scoring than other viral endpoints such as plaque formation or viral structural antigen (Vag) production. The endpoint of viral DNA synthesis has the advantage of being more closely related to DNA repair than either plaque formation or Vag production. The formation of a plaque and the synthesis of Vag both depend upon, among other processes, viral DNA repair, transcription of viral DNA, translation of viral mRNA, and synthesis of viral proteins. Viral DNA replication, however, depends upon these four processes a lesser extent than do plaque formation or Vag production. The transcription, translation and protein assembly leading to the manufacturing of late viral proteins are not required for viral DNA synthesis whereas plaque formation and Vag production both depend upon these processes. Therefore, the endpoint of viral DNA synthesis is more closely related to the process of DNA repair than most other viral endpoints.

The D_0 values calculated for HCR of UV-irradiated Ad5(LacZ) DNA synthesis in rodent complementation groups 1 through 5 range from 6.79 ± 0.98 J/m^2 for UV5 to 11.14 ± 2.00 J/m^2 for UV135 compared to 36.19 ± 4.09 J/m^2 for AA8 (Table 2). This represents reductions in viral DNA synthesis D_0 values of 3- to 5-fold compared to those in AA8. Thompson *et al.* (1980) and Busch *et al.*

(1980) have reported reductions in D_0 for CFA of UV-irradiated CHO mutants from groups 1 through 5 to be in the order of 4.25- to 5.5-fold, which are similar to the reductions in viral DNA synthesis in this study. However, the rate of incision of UV-irradiated DNA has been reported to be reduced 10- to 25-fold for these mutants, compared to parental, following a UV fluence of 6 J/m^2 to the cells (Thompson *et al.* 1982b).

This difference between reduction in CFA and DNA incision rate following UV-irradiation for the CHO mutants is likely a reflection of the two endpoints being assayed. The formation of a colony requires a minimum level of DNA repair within a finite period of time. It is this final amount of repair, and not the rate at which it is achieved, that is important for the formation of a colony. Therefore, the deficiencies in the incision rates of CHO mutants may not be a true reflection of the final amount of repair achieved and thus the reduction in incision rate may be larger than the final amount of repair achieved and consequently the reduction in CFA.

UV-sensitive CHO mutants from complementation groups 1 to 5 are deficient in the repair of the two major DNA lesions induced by ultraviolet radation, 6/4 photoproducts and cyclobutane pyrimidine dimers (PDs). UV61, a member of complementation group 6, has been shown to be deficient in the repair of PDs but is normal in its ability to repair 6/4 photoproducts (Thompson *et al.* 1989). UV survival of Ad5(LacZ) DNA synthesis in UV61 cells is reduced about 2-fold compared to AA8 (Table 3) which is similar to the 2.8-fold reduction of D_0 for CFA of UV-irradiated UV61 cells previously reported (Thompson *et al.* 1987). These smaller reductions in CFA and HCR of Ad DNA synthesis of UV61 following UV-irradiation, compared to the other UV mutants, are likely the result

of the ability of the UV61 cells to correct 6/4 photoproducts, one of the types of DNA damage induced by UV-irradiation.

The survival of Ad5(LacZ) DNA synthesis in 5-2ER2-6 cells is within the range of AA8 cells (Figure 4). This shows that the reduction in UV survival of viral DNA synthesis in UV5 cells is fully corrected by the stably transfected *ERCC2* cDNA. This is in agreement with restoration of UV resistance of UV5 cells, transfected with a full length *ERCC2* cDNA, to wild-type levels following UV-irradiation (143Weber *et al.* 1991). UV survival of Ad5(LacZ) DNA synthesis in 5T4-12 cells, which are UV5 cells stably transfected with genomic *ERCC2*, is increased to 21.71 $\pm$ 2.09 J/m^2 compared to 6.79 $\pm$ 0.98 J/m^2 for UV5 cells (Table 2). However, this increase of UV survival Ad5(LacZ) DNA synthesis in 5T4-12 cells does not achieve wild-type levels, although CFA of 5T4-12 cells following UV-irradiation has been reported to be returned to wild-type levels (Weber *et al.* 1988). This discrepancy between the two assays may be due in part to the endpoint used. Scoring viral DNA synthesis may detect a small deficiency in the rate of DNA repair that is not detected in the CFA.

The complete correction of UV survival of Ad5(LacZ) DNA synthesis in 5-2ER2-6 and the partial correction in 5T4-12 may be a reflection of the efficiency of expression of *ERCC2* in the two cell types. The number of integrated copies of *ERCC2* as well as the location of integration in the UV5 genome are two factors which may differ between these cells and contribute to the different levels of survival of Ad DNA synthesis.

UV survival of Ad5(LacZ) DNA synthesis was greater in the AA8 derived ethyl methanesulfonate-sensitive EM9 than in parental AA8 (Table 3). UV survival of Ad5(LacZ) DNA synthesis in the UCL line was markedly reduced

compared to that in the AA8 cell line. The UCL cell line obtained in our laboratory was originally thought to be the AA8-derived EM7-2 cell line which was isolated on the basis of its sensitivity to ethane methylsulfonate (Thompson *et al.* 1980). The study in which EM7-2 was isolated characterized this EMS mutant as having similar CFA following UV compared to parental AA8. However, the cell line used in this study was similar to the UV-sensitive mutants in its response to UV-irradiation (Figure 4) suggesting a possibility of mistaken identity of this line. However, it can not be ruled out that this cell line is in fact EM7-2 and that it has acquired a second mutation which renders it UV-sensitive. For the purposes of this study, this cell line has been designated unknown cell line (UCL).

The % HCR value is the D_0 for UV survival of Ad5(LacZ) DNA synthesis in a cell line expressed as a percentage of that in the appropriate parental line. The %HCR values for Ad5(LacZ) DNA synthesis of AA8 derived mutants from complementation groups 1 through 5 (Table 3) are in good agreement with the published values for reduction in CFA of these cells following UV. 4PV, 7PV, and 30PV mutants, derived from K1 parental cells, exhibit reductions in their D_0 for CFA after UV to approximately 60% to 70% of parental values (Stefanini *et al.* 1989) suggesting a small repair deficiency in these mutants compared to the UV mutants derived from AA8. Based on the results obtained from the AA8 mutants, it would be expected that K1 mutants would reactivate UV-irradiated Ad5(LacZ) to approximately 60% to 70% of K1. However, due to the large degree of variation in the data for K1 and its mutants, it was not possible to detect reductions in HCR of UV-irradiated Ad5(LacZ) in these mutants (Table 3). The large degree of variation in the data for K1 and its mutants may be a result

of a greater degree of sensitivity to culture conditions or perhaps a difference in its response to Ad infection compared to AA8 derived cells.

This study demonstrates that HCR of viral DNA synthesis is a useful assay for the detection of DNA repair deficiencies such as those present in the AA8 derived UV-sensitive mutants. Detection of smaller DNA repair deficiencies, such as those present in the K1-derived PV mutants, may be possible with a larger number of experiments or by scoring viral DNA synthesis at earlier times which would emphasize the deficiencies.

Introduction of the *denV* gene or its gene product into normal cells of human, CHO, and murine origin have been shown to increase excision repair following UV-irradiation of the cells (Yamaizumi *et al.* 1989; Kibitel *et al.* 1991; Kusewitt *et al.* 1991). Post-UV repair levels were also increased by *denV* in repair-deficient UV5 cells and XP cells from complementation groups A, B, C, D, F, and G (Valerie *et al.* 1985; Yamaizumi *et al.* 1989). Increased UV survival of UV5 and XP cells transfected with the *denV* gene was also demonstrated although wild-type levels were not achieved in either case (Valerie *et al.* 1985; Valerie *et al.* 1987). However, the introduction of *denV* did not result in increased survival of normal human, hamster, or murine cells (Kibitel *et al.* 1991; Kusewitt *et al.* 1991).

In light of these results, HCR of UV-irradiated Ad5(denV) was investigated to assess the effect of the viral encoded *denV* gene on UV survival of Ad DNA synthesis in CHO cells. In previous experiments, it was reported that *denV* was able to return the level of excision repair of UV5 cells following UV-irradiation to that of parental, but colony forming ability was only partially complemented (Valerie *et al.* 1985). Ad5(denV) infection, compared to

Ad5(LacZ) infection, resulted in increases in the D_0 values of viral DNA synthesis for cells from complementation groups 1 to 6 (Table 2). The increases in groups 1 to 4 did not return these cells to survival levels of Ad5(LacZ) infected AA8 (wild-type) which parallels the CFA results of Valerie *et al.* (1985) (Table 2). The D_0 for viral DNA synthesis in UV135 did reach wild-type levels while UV61 exhibited a more dramatic increase in survival with a D_0 of 109.15 $\pm$ 68.11 J/m^2, similar to AA8 infected with Ad5(denV) (152.97 $\pm$ 16.32 J/m^2). UV61 cells lack only the repair of PDs and not that of 6/4 photoproducts, another type of DNA damage induced by UV. The viral encoded *denV* removes the block to repair of PDs and thus UV61 exhibits a wild-type behaviour. The other mutants from groups 1 to 5 lack both the repair of PDs and 6/4 photoproducts. It is possible that the addition of the viral encoded *denV* helps UV survival but the remaining 6/4 photoproducts, which *denV* cannot incise, prevent these mutants from exhibiting UV survival levels seen in parental and UV61 cells infected with Ad5(denV). This hypothesis would predict that CFA of UV-irradiated UV61 cells which were supplied with *denV* would return to that of AA8 cells, unlike the intermediate cell survival observed by Valerie *et al.* (1985) in UV5 cells .

The *ERCC2* -transfected UV5 cell lines both increased in survival of UV-irradiated Ad5(denV) DNA synthesis in a manner similar to AA8 infected with Ad5(denV) thus demonstrating that the *ERCC2*-transfected UV5 cell lines are behaving as normal CHO cells in terms of UV survival of Ad5(denV) DNA (Table 2).

The EM9 cell line infected with Ad5(denV) behaves as a normal cell line following infection with UV-irradiated Ad5(denV) (Table 1) indicating that the mutation present in EM9 is not integral to the processing of UV-induced DNA

damage. The survival of UV-irradiated Ad5(denV) in UCL is particularly noteworthy. There is no increase in the UV survival of Ad5(denV) DNA synthesis in UCL compared to that of Ad5(LacZ). Presumably, the *denV* gene product is incising UV-damaged DNA in UCL as it appears to be doing in all the other cell lines. Thus, the lack of complementation by *denV* suggests that the mutation in UCL affects a function required following this incision event. If the mutation affected a function required prior to the incision event, the small denV protein would be able to, at least partially, circumvent this deficiency as seen in the UV-sensitive cells which are deficient in the incision step of nucleotide excision repair.

K1 and its derived mutants all exhibit increases in UV survival of Ad5(denV) DNA synthesis compared to that of Ad5(LacZ). However, the D0 value for UV survival of Ad5(denV) DNA synthesis in 7PV is not increased to the the level of that in K1 (Table 4) suggesting some repair inability in this cell line. The enhanced survival of Ad5(denV) DNA synthesis in K1 and its mutants (Table 6) is similar to that found for AA8 and its derived mutants, with the exception of the UCL line (Table5).

The transfection of the *ERCC1* gene into 43-3B cells, which belong to rodent complementation group 1, has been shown to increase unscheduled DNA synthesis following UV-irradiation or MMC treatment to levels which surpassed those of UV- or MMC-treated AA8 cells (Westerveld *et al.* 1984). Interestingly, cell survival of these *ERCC1*-transfected 43-3B cells did not attain levels equivalent to those of parental cells after these same treatments. A 1.1 kb *ERCC1* cDNA has been observed to correct the MMC- and UV-sensitivity of 43-3B cells in terms of cell survival (152van Duin *et al.* 1986). The ERCC1

correction of the UV- and MMC-sensitivities of CHO complementation group 1 cells has been demonstrated to be specific for cells from this group (van Duin *et al.* 1988a).

In view of the specific correction of the deficiencies of rodent complementation group 1 cells by *ERCC1*, an investigation was undertaken to establish the effect of the viral encoded *ERCC1* on HCR of Ad DNA synthesis following UV-irradiation of the virus. The UV survival of Ad5(ERCC1) in UV20 cells is restored to levels seen in AA8 cells infected with control virus (Table 2). However, UV survival of Ad5(ERCC1) in the mutants from complementation groups 2 to 6 is not increased compared to that for Ad5(LacZ) infections. Therefore, the results presented in this work are in agreement with published reports detailing the specificity of *ERCC1* for correction of DNA repair deficiencies of mutants belonging to rodent complementation group 1. The absence of a detectable increase in UV survival of Ad5(ERCC1) DNA synthesis in complementation groups 2 through 6 suggest that ERCC genes, 1 through 6, act in concert. ERCC1 can not compensate for a deficiency in any of the ERCC genes, 2 through 6.

The literature does not make any mention of an examination of the effect of adding exogenous *ERCC1* to repair-proficient cells. This study examines this question with the infection of parental AA8 cells with UV-irradiated Ad5(ERCC1). These experiments revealed a 2.8-fold increase in UV-survival of Ad5(ERCC1) DNA synthesis in AA8 cells compared to Ad5(LacZ) infection (Table 5). UV survival of Ad5(ERCC1) in other cell lines which are similar to AA8 in their UV survival, including *ERCC2*-transfected UV5 cell lines and the EM9 cell line, was increased to a similar extent as for that of AA8 (Table 5). This

increased survival in normal cells may be an indication that the ERCC1 protein is present in rate limiting amounts in these cells. It is also possible that human ERCC1 is more efficient than its hamster homologue and thus the addition of human ERCC1 increases UV survival of viral DNA synthesis in these cells compared to that following Ad5(LacZ) infection.

The deficiency in UV survival of viral DNA synthesis in UCL is seen to be corrected to wild-type levels after infection with UV-irradiated Ad5(ERCC1). Results of UV survival of Ad5(denV) in UCL suggest that the mutation in UCL affects a function which takes place after the incision event. The correction of UV survival of Ad DNA synthesis by *ERCC1*, coupled with the Ad5(denV) result, would suggest that*ERCC1* in addition to its function prior to or during the incision event, also has a role in the processing of UV-damaged DNA which takes place subsequent to the incision made by the denV protein. It will be important to confirm these UCL results with another independently obtained EM7-2 cell line.

The rather large D_0 values for Ad5(ERCC1) DNA synthesis, compared to those for Ad5(LacZ), in K1, 30PV, 50PV and 7PV is consistent with the hypothesis that the ERCC1 protein is acting to accelerate a rate limiting step in the processing of UV-damaged DNA (Table 4). A comparison of the D0 values for UV survival of Ad5(ERCC1) in K1 and the PV mutants reveal that 7PV and 4PV are reduced compared to K1. This observation reveals a repair deficiency for 7PV and 4PV which was not detectable following Ad5(LacZ) infection.

This study (Figure 46) and previous work in our laboratory (182Rainbow and Castillo 1992) have indicated that viral DNA synthesis for unirradiated Ad in

HeLa cells reaches a maximum between 30 and 36 hours p.i. . Based on this, 36 hours p.i. was chosen as the time point for collection of viral DNA from human cells.

The D_0 for UV survival of Ad5(LacZ) DNA replication in XP1BR group D fibroblasts was reduced to 7.36 $\pm$ 0.26 J/m^2 from 100.14 $\pm$ 30.66 J/m^2 in GM969, an apparently normal human fibroblast (Table 7). This represents a reduction in HCR for XP1BR group D of 7.3 $\pm$ 2.5% which is similar to HCR of plaque formation for XP group D observed by Day (1974). The HCR value of Vag production for UV-irradiated Ad in a XP group D fibroblast was reported to be 5-7% (Rainbow 1989). Therefore, the viral DNA synthesis assay may be used to detect repair deficiencies in a number of cell types and at the same time is less difficult and less time consuming to perform than the plaque formation assay and or the Vag assay. Zelle and Lohman (1979) have reported a 4-fold decrease in loss of endonuclease sensitive sites for XP3NE group D fibroblasts compared to normal fibroblasts. However, a direct comparison is difficult as this smaller deficiency detected in this test of XP3NE may be a result of a less severe mutation in this strain and not related to differences in the two assays.

The transformed HeLa and HT29 cell lines have previously been reported to exhibit decreased rates of incision following UV-irradiation (Squires *et al.* 1982). Although 293 cells have not been assayed in this fashion, another report has observed a repair-proficient phenotype in 293 for HCR of viral antigen production for UV-irradiated Ad (Rainbow 1989c).

An earlier investigation in this laboratory revealed that UV survival of Vag production in HeLa and HT29 cells was reduced to 30 $\pm$ 9% and 26 $\pm$ 7%, respectively, compared to normal fibroblasts (Rainbow 1989c). In this study, UV

survival of viral DNA synthesis in HeLa cells was determined to be 53.1 ± 7.7% compared to 293 (Table 3). The deficiency detected in HeLa cells with this viral DNA synthesis assay is not as great as that detected with Vag. UV survival of viral DNA synthesis in HT29 cells was reduced to 17.3 ± 1.2% compared to that of 293 cells which is similar to the magnitude of the defect detected in HT29 by Rainbow (1989c) using Vag as an endpoint. The reduction in HCR of viral DNA synthesis in HT29 is similar, or greater, than those of rodent complementation groups 1 through 6 (Table 3).

Introduction of denV into repair-proficient and repair-deficient cells of human, hamster, and murine origin results in increased UV survival of repair-deficient cells but not that of repair-proficient cells (Kibitel *et al.* 1991; Kusewitt *et al.* 1991). This differential effect of denV is paralleled by the increased UV survival of Vag production in XP group A and XP group E fibroblasts but not in normal fibroblasts following infection with Ad5(denV) compared to that of Ad5(LacZ) (Colicos *et al.* 1991).

UV survival of Ad5(denV) DNA synthesis was found to be increased in both normal GM969 fibroblasts and XP1BR group D fibroblasts (Table 7). The effect of denV on UV survival of viral functions differs when assessed by the viral DNA synthesis assay as opposed to the Vag production assay. This difference may reflect a greater sensitivity to rate of repair in the viral DNA synthesis assay compared to Vag production. Another possibility is that the MOI used in each assay plays a role as the viral DNA synthesis work was done at a MOI of 200 PFU/cell and the Vag assay was done at MOIs of less than 1 PFU/cell.

293 cells, HeLa cells, and HT29 cells all exhibited increased D_0 values for UV survival of viral DNA synthesis following Ad5(denV) infection compared to Ad5(LacZ) infection (Table 7). It would be of interest to determine if this increased UV survival of Ad5(denV) DNA synthesis would be paralleled by an increase in UV survival of plaque formation following infection of these transformed cells with Ad5(denV).

The effect of the ERCC1 protein on replication of UV-irradiated viral DNA in GM969 fibroblasts was to increase the UV survival (Table 7), as seen previously with the normal CHO cells (Table 2). This supports the view that ERCC1 protein is present in rate limiting amounts, even in normal human fibroblasts. Recently, the deficiency of XP group D cells has been shown to be corrected by *ERCC2* (Weber *et al.*, 197Flejter *et al.* 1992) and thus a study of HCR of viral DNA synthesis of an Ad vector encoding *ERCC2* would be expected to return viral DNA levels to those of wild-type. In this light, the absence of an effect of *ERCC1* on the UV survival of viral DNA synthesis in XP1BR group D fibroblasts is not unexpected since *ERCC1* has been shown to be specific in its correction of repair deficiencies in CHO mutants (van Duin *et al.* 1988a) and would presumably exhibit this specificity in human cells as well.

UV survival of Ad5(ERCC1) DNA synthesis in the transformed cells revealed increases in D_0 of approximately 1.2-, 1.5- and 2-fold in 293, HeLa, and HT29 cells, respectively (Table 7). The increases in UV survival of Ad5(ERCC1) in these cells suggests that ERCC1 is participating in a rate limiting step in the processing of UV-damaged viral DNA. The increase in D_0 for 293 cells is small compared to the increases seen in repair-proficient human fibroblasts. The constitutive expression of Ad E1A in 293 cells may be

responsible for this as excess amounts of E1A may enhance viral DNA replication. Therefore, this enhancement of viral DNA synthesis due to E1A may serve to mask the effect of ERCC1 on UV survival of viral DNA synthesis.

The increases in viral DNA synthesis of UV-irradiated Ad5(ERCC1) in HeLa and HT29 are consistent with the view that ERCC1 is able to participate in a rate limiting step in the DNA repair process. Thus, *ERCC1* is acting to complement the decrease in the incision rate previously reported for these cells (Squires *et al.* 1982).

A comparison of the enhancement of UV survival of Ad5(ERCC1) DNA synthesis in HeLa, HT29, and the CHO mutants reveals that ERCC1 helps HeLa, HT29, and members of rodent complementation group 1, but not groups 2 through 6. This suggests that the deficiencies which lie in HeLa and HT29 are not similar to those that are found in rodent groups 2 through 6. However, it remains possible that the HeLa and HT29 repair deficiencies are similar to the one found in rodent complementation group 1 or that they are some other deficiencies unrelated to those discovered in CHO mutants.

Survival of Viral DNA Synthesis for γ-Irradiated Adenovirus in CHO Cells

The spectrum of DNA damage induced by γ-rays is different from that induced by UV irradiation. The conditions under which the γ-irradiation is carried out influences this spectrum of DNA damage. Of particular interest are the results of Bennett and Rainbow (1989a) who found HCR of Ad in an XP fibroblast to be 57% of normal when the virus was irradiated at -75^{0}C whereas

the HCR was much closer to wild-type (88%) if the virus was irradiated at 0^0C. Therefore, HCR of γ-irradiated Ad5(LacZ), Ad5(denV) and Ad5(ERCC1) was investigated in CHO mutants to determine the responses of both the mutants and the two DNA repair genes to this different spectrum of UV damage.

γ-ray survival of Ad5(LacZ) DNA synthesis in parental AA8 cells was found to have a D_0 value of 437.27 ± 34.77 krads (Table 9). This is significantly larger than 99 ± 14 krads, the D_0 for plaque formation of γ-ray treated Ad 2 in human KB cells reported by Bennett and Rainbow (1989). Reductions in D_0 values for Ad5(LacZ) DNA synthesis following γ-irradiation of the virus for all the CHO mutants tested, as seen in Table 9, revealed UV5 to be significantly reduced to 41% of AA8 values while EM9 exhibited a D_0 corresponding to 77% of wild-type values. The reductions in HCR of viral DNA synthesis of UV20 and UV41 were found to be between those of UV5 and EM9. These results are consistent with previous work which demonstrated a degree of overlap between the repair pathway for UV-induced PDs and that of γ-ray induced DNA damage (Chan and Little 1981).

A significant increase in D_0 values for UV survival of Ad5(denV) DNA replication following γ-ray treatment of the virus, relative to those for Ad5(LacZ), were seen for all cell lines tested, except UV20 (Table 9). This lack of enhanced γ-ray survival of Ad5(denV) DNA synthesis in UV20 suggests that ERCC1 is required for the denV-mediated repair which is seen to take place in the other mutants. The small increases for γ-ray survival of viral DNA synthesis in the Ad5(denV) infections, coupled with the small decreases in HCR Ad5(LacZ) DNA synthesis, support the hypothesis that some of the damage

induced in the viral DNA by γ-irradiation at 0°C is UV-like as the denV protein is thought to be specific for PDs (Nakabeppu *et al.* 1982; Gordon and Haseltine 1980). The other possibility which must be considered is that the enhanced replication of viral DNA synthesis (seen in Figure 46) in AA8 cells infected with Ad5(denV) compared to that of Ad5(LacZ) shifts the time course of viral DNA synthesis such that any deficiency in repair of Ad5(denV) would not be as pronounced.

The D_0 values for Ad5(ERCC1) DNA synthesis of γ-ray treated virus were increased in all of the mutants that were investigated (Table 9). The absolute increases in HCR of viral DNA synthesis following γ-irradiation were greater than that of Ad5(denV) suggesting that *ERCC1*, in addition to its involvement in the repair of UV-like DNA damage, is also implicated in the repair of other types of γ-ray induced DNA damage.

Adenovirus Replication in Mammalian Cells

Recent reports in the literature have indicated a dual involvement of some proteins in the repair of DNA damage as well as replication of undamaged DNA. This link between DNA repair and DNA replication has been established for both human single stranded DNA binding protein (hssb) and proliferating cell nuclear antigen (PCNA) (Coverley *et al.* 1991; Shivji *et al.* 1992). It was therefore considered of interest to examine the viral DNA replication capabilities of the parental and mutant CHO cells as well as those of a number of human cell lines. The effect of two known DNA repair genes, *denV*

and *ERCC1*, on the replication of viral DNA in these cell lines was also investigated.

DNA repair mutants UV20, UV24, and UV61 are reduced in the amount of Ad5(LacZ) DNA synthesized by 72 hours p.i. compared to AA8 (Table 11). This raises the possibility that *ERCC1*, *ERCC3*, and *ERCC6*, the three human genes which have been shown to complement the DNA repair deficiencies of UV20, UV24, and UV61, respectively (Westerveld *et al.* 1984; Weeda *et al.* 1990; Troelstra *et al.* 1990), may also be involved in DNA replication. ERCC1 has DNA binding domains, shown to be necessary for its DNA repair role, which could also be important in a possible DNA replication role. Both ERCC3 and ERCC6 contain conserved regions which are associated with helicase activities and thus it is not unlikely that these two repair genes may be involved in DNA replication (Weeda *et al.* 1990; Troelstra *et al.* 1990).

The PV mutants derived from K1, with the exception of 7PV, are all within the wild-type range for replication of Ad5(LacZ) DNA (Table 12). These observations would seem to indicate that the gene product which are altered in 7PV may be involved in DNA replication whereas those gene products which are altered in the other PV mutants are unlikely to play a role in DNA replication. Alternatively, it is possible that, as in the case of viral DNA repair in these cells, the defects present in 30PV, 50PV, and 4PV are too small to be detected with this viral DNA synthesis assay.

The effect of Ad5(denV) infection, relative to that of Ad5(LacZ), on viral DNA synthesis in parental and mutant CHO cells was investigated. The

replication enhancement factor (REF), defined as the ratio of replication of Ad5(denV) DNA at 72 hours p.i. to that of Ad5(LacZ) DNA during the same time period, was used as the measure of comparison for this study.

The Ad5(denV) REFs for AA8 and all its derived cell lines are given in Table 13. Viral DNA replication was found to be significantly increased by *denV* in AA8 and all of its derived cells which were examined. K1 and its derived cells also have significantly increased levels of Ad5(denV) DNA replication compared to those following Ad5(LacZ) infection (Table 14).

These results suggest that the denV product is somehow enhancing the replication of viral DNA in CHO cells. Since CHO cells are semi-permissive for Ad infection, the question arises as to whether the denV protein, which is known to possess DNA binding properties, is able to improve the efficiency of some step of the CHO-Ad interaction during infection. One of the steps that occurs during a permissive Ad infection is the binding of the cellular protein p53 by Ad E1B. Recently, a model of mammalian DNA repair has been put forward in which p53 acts to arrest the cell cycle following DNA damage by a variety of agents (Lane 1992; Kastan *et al.* 1991). The model also suggests that the binding of p53 during Ad infection is necessary to prevent arrest of the cell cycle so that viral DNA may be replicated. It will be of interest to determine whether hamster p53 and human p53 are sufficiently different to explain the semi-permissive nature of CHO-Ad infections. In the event that the E1B-p53 interaction is less stable in hamster cells, it is possible that accumulation of p53 is responsible, at least in part, for the decreased levels of Ad DNA replication following Ad5(LacZ) infection. The addition of denV to the CHO cells via Ad5(denV) is somehow acting to overcome the reduction in viral DNA synthesis

and it is possible that the denV protein is acting to stabilize the E1B-p53 interaction.

The effect of the Ad-encoded *ERCC1* gene on replication of viral DNA in CHO cells was assessed in the same manner as that of *denV*. The results of this study were that the replication of Ad5(ERCC1) DNA is significantly increased compared to that for Ad5(LacZ) in all of the CHO cells examined (Table 13 and Table 14). This general effect of *ERCC1* on the replication of viral DNA in a range of CHO cells would suggest that this DNA repair protein is also able to complement a viral DNA replication deficiency in CHO cells. The means by which ERCC1 is able to complement viral DNA replication in CHO cells may be by stabilizing the p53-E1B interaction, similar to that proposed for denV. Another possibility is that ERCC1 acts directly at the level of DNA replication. The recent discoveries of the involvement of human single-stranded DNA binding protein (hssb) and proliferating cell nuclear antigen (PCNA), two known DNA replication proteins, in the excision repair of DNA is consistent with the hypothesis that ERCC1 is required in both DNA repair and DNA replication (Coverley et al. 1991; Shivji et al. 1992).

Previous work in our laboratory and this study (Figure 46) have indicated that viral DNA synthesis for unirradiated virus reaches a maximum in human cells between 30 and 36 hours p.i. (Rainbow and Castillo 1992). Based on this information, 36 hours p.i. was chosen as the time point for collection of viral DNA from human cells.

The DNA repair-deficient XP1BR group D fibroblasts demonstrate a reduction in the amount of viral DNA synthesized by 36 hours p.i., relative to normal GM969 fibroblasts (Table 15). The recent observations that the repair deficiency in XP group D cells is corrected by *ERCC2* (Weber *et al.* 1992; Flejter *et al.* 1992) might suggest that this DNA repair gene in also involved in DNA replication as well. The existance of a high degree of homology between ERCC2 and the yeast RAD3 protein, which possesses ATP dependent helicase activity, is also consistent with the hypothesis that ERCC2 plays a role in DNA replication (Weber *et al.* 1990). Interestingly, the CHO cell line UV5 which is complemented by ERCC2 in terms of DNA repair was not found to be deficient in viral DNA replication (Table 11). One explanation for these results is that the ERCC2 mutation in the UV5 cell line only affects a region of the protein which is involved in the repair of DNA. Consequently, its DNA replicative abilities are normal. Another possibility may be that human ERCC2 has an additional DNA replication function whereas its hamster homologue does not.

The colon carcinoma cell line, HT29, is also reduced in its ability to replicate Ad5(LacZ) DNA compared to the virally transformed 293 cells (Table 15). This result suggests that the alteration in HT29 which affects the incision rate of damaged DNA also plays a role in DNA replication.

The effect of Ad5(denV) infection, relative to that of Ad5(LacZ), on viral DNA synthesis in fibroblasts and tumour-derived cells of human origin was investigated. The ratio of replication of Ad5(denV) DNA to that of Ad5(LacZ) for a cell line was defined as the replication enhancement factor (REF). This gives

an indication of the ability of the repair gene to augment the viral DNA replication capabilities of a cell line.

The Ad5(denV) REFs for normal GM969 fibroblasts and repair-deficient XP1BR group D fibroblasts are found to be increased significantly (Table 16). Interestingly, the transformed 293 cell line exhibits a significant reduction in Ad5(denV) DNA replication compared to that following Ad5(LacZ). HeLa and HT29 cells both exhibit significant increases in the REF for Ad5(denV).

Taken together, these results suggest that the factor/function that denV is able to complement in CHO cells is also complemented to a certain extent in human cells.

Human XP1BR group D fibroblasts and human HT29 cells were found to exhibit increased Ad DNA replication following Ad5(ERCC1) infection compared to that following Ad5(LacZ) infection (Table 16). An interesting observation is that Ad5(ERCC1) REF is significantly reduced in 293 cells raising the possibility that large amounts of ERCC1 may be detrimental to cells. This is consistent with the observations that ERCC1 appears to be rapidly degraded in cells (van den Berg *et al.* 1991). If large quantities of ERCC1 are detrimental to cells, it would seem important that the cell be capable of rapidly degrading the protein, if need be. It is also possible that the reduction in Ad5(denV) DNA synthesis and Ad5(ERCC1) DNA synthesis compared to that of Ad5(LacZ) in 293 cells may partially be the result of the constitutive expression of E1A by 293 cells. The reason for this is unclear, however it is very interesting that 293 cells are the only cells examined which exhibit a reduction in viral DNA synthesis

following infection with Ad5(denV) or Ad5(ERCC1) compared to that following infection with Ad5(LacZ).

The results presented regarding replication of unirradiated viral DNA suggest a role for *denV* and *ERCC1* in viral DNA replication. It must be considered that these proteins may not be involved in the actual process of DNA replication but that they are able to alter the kinetics of viral infection and thereby indirectly affect viral DNA replication.

Adenoviral DNA Replication in Human, Hamster, and Murine Cells

The possibility that foreign genes alter the level of permissiveness of Ad infections is an important consideration for Ad related research. For instance, the development of Ad vaccines with increased permissiveness may be an important consideration for inducing a stronger immune response in a vaccinated organism.

The first step taken to address this issue was a comparison of the viral DNA replication kinetics in HeLa, AA8, and mouse L cells infected with the three recombinant viruses. The onset of Ad5(LacZ) DNA replication in HeLa cells appears to be approximately 10 hours post-infection (Figure 46) which is about the time of onset of wild-type viral DNA replication in HeLa cells reported in the literature (for review see Horwitz 1990). Onset of Ad5(LacZ) DNA synthesis in AA8 cells is delayed compared to HeLa as the viral DNA production begins between 20 and 24 hours p.i. (Figure 46). This result agrees with observations

reported by Longiaru and Horwitz (1981) in that they found viral DNA synthesis in CHO is delayed compared to human cells. Their observations differ, however, from those of this study as they found the delay is overcome by 40 hours p.i. The reason for this discrepancy is not apparent as the MOI used in this study of 40 PFU/cell is approximately 5600 virions/cell (see Table 11). This is similar to the input MOI used in the Longiaru and Horwitz (1981) study as they used an input MOI of 4000 virions per cell.

Ad replication in L cells is restricted to a much greater extent that in AA8 as viral DNA in L cells infected with control Ad5(LacZ) do not rise above input values at any time point in the investigation (Figure 46).

The denV protein does not increase the permissive nature of HeLa cells in terms of viral DNA replication kinetics (Figure 47) although AA8 cells do appear to be helped by this protein. This would suggest that the Ad DNA replicating function or factor which is deficient in AA8 is partially overcome by the addition of *denV* to these cells. This replication factor/function is fully functional in HeLa and so the denV protein can not act to increase its efficiency. Viral DNA replication in L cells also appears to be helped in a small way by the addition of the denV protein.

The viral DNA kinetics for Ad5(ERCC1) DNA replication in HeLa, AA8, and L cells are altered compared to those of Ad5(LacZ) in the same fashion as for Ad5(denV) (Figure 48). ERCC1 appears to help a factor/function which is deficient in AA8 cells but does not improve the performance of this factor in

HeLa. As with Ad5(denV), L cells replicate increased amounts of viral DNA following Ad5(ERCC1) infection compared to that of Ad5(LacZ).

These foreign repair genes were not observed to enhance viral DNA replication in permissively infected HeLa cells. However, viral DNA replication is helped by *denV* and *ERCC1* in the semi-permissively infected AA8 cells. This suggests that both proteins are acting to compensate for a factor/function which is present in HeLa but reduced in AA8. This particular factor which seems to be greatly reduced in L cells is only restored to a small degree by the repair proteins.

Adenoviral Protein Synthesis in Human, Hamster, and Murine Cells

Previous reports have observed that the synthesis of viral proteins in cells which were not permissive for Ad infection were altered compared to permissive infections (Longiaru and Horwitz 1981; Radna *et al.* 1987; Eggerding and Pierce 1986). These observations coupled with the changes in viral DNA kinetics as a result of *denV* and *ERCC1* prompted an investigation of viral protein synthesis in HeLa, CHO, and mouse L cells infected with the three recombinant viruses. This study examined the production of viral structural proteins, the Ad DNA binding protein, and E1A protein following infections with Ad5(LacZ), Ad5(denV), and Ad5(ERCC1) in HeLa, CHO, and L cells.

An examination of the time course of viral structural protein synthesis in HeLa, AA8, and L cells infected with control Ad5(LacZ) reveal reduced amounts

of Vag produced in AA8 compared to HeLa and no detectable Vag produced in L cells (Figure 49). The rates of synthesis of Ad DBP appear to be similar in HeLa and AA8 although the onset of its synthesis is delayed in AA8, in agreement with observations by Eggerding and Pierce (1986).

Ad5(denV) infection of HeLa cells causes the onset of hexon synthesis to be brought forward, although the kinetics of all the other Vag appear similar to Ad5(LacZ) infection (Figure 50). The presence of *denV* also hastens the onset of synthesis of Vag in AA8 cells and increases the rates of synthesis of hexon and fibre as well. Ad5(denV) infected AA8 cells synthesize Ad DBP earlier than do Ad5(LacZ) infected AA8 cells. These observations in HeLa and AA8 suggest a link between the factor/function in AA8 cells which *denV* renders more efficient and both the synthesis of early proteins (Ad DBP) and late proteins (Vag). A slight increase in the permissivity of L cell infections is seen following Ad5(denV) infection with the production of greater amounts of Ad DBP at earlier times and the synthesis of some hexon, which is not seen in the Ad5(LacZ) infection.

The effects of the denV protein on the production of Vag and Ad DBP in AA8 and L cells are also seen in the Ad5(ERCC1) infections of these cells (Figure 51). The increased amounts of the Vag and earlier onset of synthesis of these proteins are also brought about through the action of ERCC1. The HeLa infection with Ad5(ERCC1) is interesting as no Vag or Ad DBP are detectable after 24 hours p.i. (Figure 51). These observations, coupled with the significant

decrease in the Ad5(ERCC1) REF for 293 cells, is evidence that large amounts of ERCC1 are detrimental to cells.

A specific comparison of the amounts of Ad DBP was then done by using a monoclonal antibody directed against this viral protein (Figure 52). The comparison times chosen were 12 hours for HeLa and AA8 and 24 hours for L cells since these are the times when Ad5(LacZ) infection appears to be lagging behind those of Ad5(denV) and Ad5(ERCC1). The rates of synthesis of Ad DBP at these comparison times correlate with the amounts of Vag being produced at those times.

The amount the early Ad protein E1A being produced at these same comparison times was then investigated (Figure 53). The most notable difference is that HeLa cells are producing greater quantities of E1A than either AA8 or L cells and that three differentially phophorylated species of E1A are being made in HeLa while only the largest species is detectable in the AA8 and L cells. From this figure, it would seem that the differences in Vag and Ad DBP production at these times are not related solely to the presence of E1A. Approximately equal amounts of E1A are produced by each virus in the each of the cell lines at the comparison times, yet the amounts of Vag and Ad DBP produced are seen to vary.

UV Enhanced Reactivation of UV-irradiated Adenovirus in CHO cells

Inducible DNA repair genes in bacteria are under control of the RecA-LexA regulon. DNA damage inducible repair pathways also exist in mammalian cells and recently the anti-oncogene p53 has been proposed to be involved in their regulation (Lane 1992).

Several lines of evidence support the hypothesis that p53 is involved in the regulation of DNA repair. Firstly, the level of p53 increases in the cell subsequent to UV-irradiation, treatment with radiomimetic drugs, γ-irradiation, or treatment with various chemotherapeutic drugs that damage DNA (Maltzman and Czyzyk 1984; Kastan *et al.* 1991). Kastan *et al.* (1991) have demonstrated that it is this accumulation of p53 which mediates arrest of the cell cycle in G1 following DNA damage. Therefore, p53 acts in an analogous manner to RecA-LexA following DNA damage in that it acts to arrest the cell cycle. This arrest stops DNA replication which allows time for the repair of DNA damage. The importance of this arrest to allow DNA repair is evident in transgenic mice which are deficient for p53. These mice develop normally but are very prone to spontaneous tumours, presumably as a result of mutations which occur due to the lack of regulation of DNA repair.

In addition to its role in G1 arrest, wild-type p53 has also been shown to be a transcriptional activator (Scharer and Iggo 1992; Kern *et al.* 1992). Although there is yet no evidence to suggest that p53 acts directly to induce transcription of specific mammalian DNA repair genes as does cleavage of

LexA in *E. coli*, it is a very interesting hypothesis that increased levels of p53 may act to induce transcription of DNA repair genes.

Treatment of cells with DNA damaging agents prior to viral infection is a means of probing the inducible repair pathways of cells, proposed to be regulated by p53, by monitoring the enhanced reactivation of viral functions. There have not been any Ad enhanced reactivation studies of CHO cells to date primarily because these cells do not yield infectious virus when infected with Ad. Therefore, plaque formation, one of the most common endpoints used in virus-cell interaction studies, is not a viable option for CHO Ad infections. CHO cells have been shown, however, to replicate Ad DNA and this study utilized this viral function as an endpoint to examine inducible repair phenomena in CHO cells.

An examination of UVER of Ad DNA synthesis in AA8 cells UV-irradiated immediately prior to infection reveals a small positive UVER value with a UV fluence of 15 J/m2 to the cells if viral DNA is scored at 74 hours p.i. but not at 49.5 hours p.i. (Figure 56a; Figure 56b). This dependency on time of scoring parallels UVER results in normal and AT fibroblasts reported by Bennett and Rainbow (1988a). They observed higher UVER of Vag production when scoring was performed at approximately 70 hours p.i. compared to earlier times. Another interesting parallel between these two studies is the similar time course of UVER in CHO and AT cells. This study observed a negative UVER for AA8 cells when time of scoring was 49.5 hours p.i. Bennett and Rainbow (1988a) report that UVER of Vag in AT cells is also negative when scoring is done at 30 and 50 hours p.i. but becomes positive at times subsequent to 60 hours p.i.

Delaying Ad infection for a period of 12 hours following UV-irradiation of normal human cells results in a greater UVER compared to immediate infection (Bennett 1986). UVER values for delays greater than 12 hours are found to return to levels similar to, or less than, that for immediate infection. This study found that delaying infection of CHO cells with Ad for a period of 12 hours subsequent to UV-irradiation of the cells resulted in increased UVER of Ad DNA synthesis. Unfortunately, delaying infection of CHO cells for periods greater than 12 hours subsequent to UV-irradiation of the cells was not possible as the cell sheet began to lift off shortly after this time.

REFERENCES

Arikan, E., Kulkarni, M. S., Thomas, D. C., and Sancar, A. (1986). Sequences of the E. coli uvrB gene and protein. Nucleic Acids Res. **14**, 2637.

Arrand, J. R. and Roberts, R. J. (1973). The nucleotide sequences at the termini of adenovirus 2 DNA. J. Mol. Biol. **128**, 577.

Auerbach, A. D. and Wolman, S. R. (1978). Susceptibility of Fanconi's anemia fibroblasts to chromosome damage by carcinogens. Nature. **261**, 494.

Bachenheimer, S. and Darnell, J. E. (1975). Adenovirus 2 mRNA is transcribed as part of a high molecular weight precursor RNA. Proc. Natl. Acad. Sci. (USA). **72**, 4445.

Backendorf, C., Spaink, H., Barbeiro, A. P., and van de Putte, P. (1986). Structure of the uvrB gene of Escherichia coli. Homology with other DNA repair enzymes and characterization of the uvrB5 mutation. Nucleic Acids Res. **14**, 2877.

Bagg, A., Kenyon, C. J., and Walker, G. C. (1981). Inducibility of a gene product required for UV and chemical mutagenesis in Escherichia coli. Proc. Natl. Acad. Sci. (USA). **78**, 5749.

Bennett, C.B. (1986). UV enhanced mutagenesis of adenovirus in human fibroblasts. Ph.D. thesis. Department of Biology, McMaster University, Hamilton, Ontario.

Bennett, C. B. and Rainbow, A. J. (1988a). Enhanced reactivation and mutagenesis of UV-irradiated adenovirus in normal human fibroblasts. Mutagenesis. **3**, 157.

Bennett, C. B. and Rainbow, A. J. (1988b). Delayed expression of enhanced reactivation and decreased mutagenesis of UV-irradiated adenovirus in UV-irradiated ataxia telangiectasia fibroblasts. Mutagenesis. **3**, 389.

Bennett, C. B. and Rainbow, A. J. (1989). DNA damage and biological expression of adenovirus: A comparison of liquid versus frozen conditions of exposure to γ rays. Radiat. Res. **120**, 102.

Berget, S. M., Moore, C., Sharp, P. A. (1977). Spliced segments at the 5' terminus of adenovirus 2 late mRNA. Proc. Natl. Acad. Sci. (USA). **74**, 3171.

Berkner, K. L. (1988). Development of adenovirus vectors for the expression of heterologous genes. Biotechniques. **6**, 616.

Blancou, J., Kieny, M. P., Lathe, R., Lecoq, J. P., Pastoret, P. P., Soulebot, J. P., and Desmettre, P. (1986). Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. Nature. **322**, 373.

Brent, R., and Ptashne, M. (1981). Mechanism of action of the lexA gene product. Proc. Natl. Acad. Sci. (USA). **78**, 4204.

Bockstahler, L. E. and Lytle, C. D. (1970). Ultraviolet light-enhanced reactivation of a mammalian virus. Biochem. Biophys. Res. Comm. **41**, 184.

Bohr, V. A., Chu, E. H. Y., van Duin, M., Hanawalt, P. C., and Okumoto, D. S. (1988). Human repair gene restores normal pattern of preferential DNA repair in repair defective CHO cells. Nucleic Acids Res. **16**, 7397.

Bohr, V. A. and Hanawalt, P. C. (1987). Enhanced repair of pyrimidine dimers in coding and non-coding genomic sequences in CHO cells expressing a prokaryotic DNA repair gene. Carcinogenesis. **8**, 1333.

Bohr, V. A., Okumoto, D. S., and Hanawalt, P. C. (1986). Survival of UV-irradiated mammalian cells correlates with efficient DNA repair in an essential gene. Proc. Natl. Acad. Sci. (USA). **83**, 3830.

Bohr, V. A., Smith, C. A., Okumoto, D. S., and Hanawalt, P. C. (1985). DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. Cell. **40**, 359.

Busch, D. B., Cleaver, J. E., and Glaser, D. A. (1980). Large-scale isolation of UV-sensitive clones of CHO cells. Somatic Cell Genet. **6**, 407.

Busch, D., Greiner, C., Lewis, K., Ford, R., Adair, G., and Thompson, L. H. (1989). Summary of complementation groups of UV-sensitive CHO cell mutants isolated by large-scale screening. Mutagenesis. **4**, 349.

Carlin, C. R., Tollefson, A. E., Brady, H. A., Hoffman, B. L., Ranheim, T. S., and Wold, W. S. M. (1989). Epidermal growth factor receptor is down-regulated by a 10,400 MW protein encoded in E3 of adenovirus. Cell. **57**, 135.

Caron, P. R., Kushner, S. R., and Grossman, L. (1985). Involvement of helicase II (uvrD gene product) and DNA polymerase I in excision mediated by the UvrABC protein complex. Proc. Natl. Acad. Sci. (USA). **82**, 4925.

Challberg, M. D. and Kelly, T. J. Jr. (1979). Adenovirus DNA replication in vitro. Proc. Natl. Acad. Sci. (USA). **76**, 655.

Chan, G. L. and Little, J. B. (1981). Cross sensitivity of certain xeroderma pigmentosum and Cockayne syndrome fibroblast strains to both ionising radiation and ultrviolet light. Mol. Gen. Genet. **181**, 562.

Cleaver, J.E. (1968). Defective repair replication of DNA in xeroderma pigmentosum. Nature. **218**, 652.

Cleaver, J. E. and Bootsma, D. (1975). Xeroderma pigmentosum: biochemical and genetic characteristics. Ann. Rev. Genet. **9**, 19.

Colicos, M. A. (1988). Construction of an adenovirus expression vector containing the T4 denV gene, which can complement the DNA repair deficiency of xeroderma pigmentosum fibroblasts. M. Sc. thesis, McMaster University, Hamilton, Ontario.

Colicos, M. A., Haj-Ahmad, Y., Valerie, K., Henderson, E. E., and Rainbow, A. J. (1991). Construction of a recombinant adenovirus containing the denV gene from bacteriophage T4 which can partially restore the DNA repair deficiency in xeroderma pigmentosum fibroblasts. Carcinogenesis. **12**, 249.

Collins, A. and Johnson, R. T. (1987). DNA repair mutants in higher eukaryotes. J. Cell Sci. Suppl. **6**, 61.

Coohill, T. P. (1981). Mammalian cell viral capacity: an alternative assay for ultraviolet radiation damage. Photochem. Photobiol. **34**, 525.

Coohill, T. P. (1986). Virus-cell interactions as probes for vacuum-ultraviolet radiation damage and repair. Photochem. Photobiol. **44**, 359.

Coverley, D., Kenny, M. K., Munn, M., Rupp, W. D., Lane, D. P., and Wood, R. D. (1991). Requirement for the replication protein SSB in human DNA excision repair. Nature. **349**, 538.

Cutt, J. R., Shenk, J., and Hearing, P. (1987). Analysis of adenovirus early region-4 encoding polypeptides sythesized in productively infected cells. J. Virol. **61**, 543.

D'Ambrosio, S. M. and Setlow, R. B. (1976). Enhancement of postreplication repair in Chinese hamster cells. Proc. Natl. Acad. Sci. (USA). **73**, 2396.

Davidson, D. and Hassell, J. A. (1987). Overproduction of polyomavirus middle T antigen in mammalian cells through the use of an adenovirus vector. J. Virol. **61**, 1226.

DasGupta, U. B. and Summers, W. C. (1978). Ultraviolet reactivation of herpes simplex virus is mutagenic and inducible in mammalian cells. Proc. Natl. Acad. Sci. (USA). **75**, 2378.

Day, R. S. III. (1974). Studies on repair of adenovirus 2 by human fibroblasts using normal, xeroderma pigmentosum, and xeroderma pigmentosum heterozygous strains. Cancer Res. **34**, 1965.

Day, R. S. III. (1979). Human brain tumour cell strains with deficient host-cell reactivation of N--methyl-N'-nitro-N-nitrosoguanidine-damaged adenovirus 5. Nature. **279**, 797.

Defais, M. J., Hanawalt, P. C., and Sarasin, A. R. (1983). Viral probes for DNA repair. Adv. Radiat. Biol. **10**, 1.

Dewar, R. L., Natarajan, M. B., Basudevachari, M. B., and Salzman, N. P. (1989). Synthesis and processing of human immunodeficiency virus type 1 envelope proteins encoded by a recombinant human adenovirus. J. Virol. **63**, 129.

Doolittle, R. F., Johnson, M. S., Husain, I., Van Houten, B., Thomas, D. C., and Sancar, A. (1986). Domainal evolution of a prokaryotic DNA-repair protein: relationship to active transport proteins. Nature. **323**, 451.

Dowd, D. R. and Lloyd, R. S. (1990). Biological significance of facilitated diffusion in protein-DNA interactions: Application to T4 endonuclease V-initiated DNA repair. J. Biol. Chem. **265**, 3424.

Dulbecco, R. (1949). Reactivation of ultraviolet inactivated bacteriophage by visible light. Nature. **163**, 949.

Dulhanty, A. M., Rubin, J. S., and Whitmore, G. F. (1988). Complementation of the DNA-repair defect in a CHO mutant by human DNA that lacks highly abundant repetitive sequences. Mutat. Res. **194**, 207.

Eggerding, F. A. and Pierce, W. C. (1986). Molecular biology of adenovirus type 2 semipermissive infections I. Viral growth and expression of viral replicative functions during restricted adenovirus infection. Virology. **148**, 97.

Flejter, W. L., McDaniel, L. D., Johns, D., Friedberg, E. C., and Schultz, R. A. (1992). Correction of xeroderma pigmentosum complementation group D mutant cell phenotypes by chromosome and gene transfer: Involvement of the human ERCC2 DNA repair gene. Proc. Natl. Acad. Sci. (USA). **89**, 261.

Friedberg, E. C. (1985). DNA Repair. W. H. Freeman and Company, Ron Newcomer and Associates, San Francisco.

Friedberg, E. C. and King, J. J. (1971). Dark repair of ultraviolet-irradiated deoxyribonucleic acid by bacteriophage T4: purification and characterization of a dimer-specific phage-induced endonuclease. J. Bacteriol. **106**, 500.

Friefeld, B. R., Lichy, J., Hurwitz, J., and Horwitz, M. S. (1983). Evidence for an altered adenovirus DNA polymerase in cells infected with mutant H5ts149. Proc. Natl. Acad. Sci. (USA). **80**, 1589.

Garon, C. F., Berry, K. W., and Rose, J. A. (1972). A unique form of terminal redundancy in adenovirus DNA molecules. Proc. Natl. Acad. Sci. (USA). **69**, 2391.

Gates, F. L. (1930). A study of the bactericidal action of ultraviolet light. III. The absorption of ultraviolet light by bacteria. J. Gen. Physiol. **14**, 31.

Geleziuna, R., McQuillan, A., Malapetsa, A., Hutchinson, M., Kopriva, D., Wainberg, M. A., Hiscott, J., Bramson, J., and Panasci, L. (1991). Increased DNA synthesis and repair-enzyme expression in lymphocytes from patients with chronic lymphocytic leukemia resistant to nitrogen mustards. J. Natl. Cancer Inst. **8**, 557.

German, J., Bloom, D., and Passarge, E. (1977). Bloom's syndrome. V. Surveillance for cancer in affected families. Clin. Genet. **12**, 162

Ghosh-Choudhury, G. and Graham, F. L. (1987). Stable transfer of a mouse dihydrofolate reductase gene into a deficient cell line using human adenovirus vector. Biochem. Biophys. Research Comm. **147**, 964.

Gill, R. F. and Coohill, T. P. (1987). A comparison of mammalian cell sensitivity to either 254 nm or artificial "solar" simulated radiation. Photochem. Photobiol. **45**, 264.

Gooding, L. R., Elmore, L. E., Tollefson, A. E., Brody, H. A., and Wold, W. S. M. (1988). A 14,700 MW protein from the E3 region of adenovirus inhibits cytolysis by tumor necrosis factor. Cell. **53**, 341.

Gordon, L.K. and Haseltine, W.A. (1980). Comparison of the cleavage of pyrimidine dimers by the bacteriophage T4 and *Micrococcus luteus* UV-specific endonucleases. J. Biol. Chem. **255**, 12047.

Graham, F. L., Abrahams. P. J., Mulder, C. et al (1974). Studies on in vitro transformation by DNA and DNA fragments of human adenoviruses and SV40. Cold Spring Harbor Symp. Quant. Biol. **39**, 637.

Graham, F. L. and Prevec, L. (1991). Manipulation of adenovirus vectors. In Methods in Molecular Biology, Vol. 7: Gene Transfer and Expression Protocols (ed. E. J. Murray). pp 109. The Humana Press Inc. Clifton, NJ.

Graham, F. L., Smiley, J., Russell, W. C., and Nairn, R. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. **36**, 59.

Gudas, L. J. (1976). The induction of protein X in DNA repair and cell division mutants of Escherichia coli. J. Mol. Biol. **104**, 567.

Hall, J. D. and Mount, D. W. (1981). Mechanisms of DNA replication and mutagenesis in ultraviolet-irradiated bacteria and mammalian cells. Prog. Nucleic Acid Res. Mol. Biol. **25**, 53

Hanawalt, P. C. (1987). Preferential DNA repair in expressed genes. Environ. Health Persp. **76**, 9.

Harlow, E., Franza, B. R. Jr., and Schley, C. (1985). Monoclonal antibodies specific for adenovirus early region 1A proteins: Extensive heterogeneity in early region 1A products. J. Virol. **55**, 533.

Hellman, K. B., Haynes, K. F., and Bockstahler, L. E. (1974). Radiation-enhanced survival of a human virus in normal and malignant rat cells. Proc. Soc. Exp. Biol. Med. **145**, 255.

Hirschi, M., Netrawali, M. S., Remson, J. F., and Cerutti, P. A. (1981). Formation of DNA single-strand breaks by near-ultraviolet and γ-rays in normal and Bloom's syndrome skin fibroblasts. Cancer Res. **41**, 2003.

Hirt, B. (1967). Selective extraction of polyoma DNA from mouse cell cultures. J. Mol. Biol. **26**, 365.

Hoeijmakers, J. H. J. (1991). How relevant is the Escherichia coli UvrABC model for excision repair in eukaryotes? J. Cell Sci. **100**, 687.

Hoeijmakers, J. H. J., Odijk, H., and Westerveld, A. (1987). Differences in the amount of transfected, intergrated DNA between various rodent and human cell lines. Exp. Cell Res. **169**, 111.

Horne, R. W., Bonner, S., Waterson, A. P., and Wildy, P. (1959). The icosahedral form of adenovirus. J. Mol. Biol. **1**, 84.

Horwitz, M. S. (1971). Intermediates in the replication of type 2 adenovirus DNA. Virology. **8**, 675.

Horwitz, M. S. (1990). Adenoviridae and their replication. In Virology, 2nd Edition (ed B. N. Fields). pp. 1679. Raven Press, New York.

Horwitz, M. S., Brayton, C., and Baum, S. G. (1973). Synthesis of type 2 adenovirus DNA in the presence of cycloheximide. J. Virol. **11**, 544.

Howard-Flanders, P. (1981). Inducible repair of DNA. Sci. Am. **245**, 72.

Howard-Flanders, P., Boyce, R. P., and Theriot, L. (1966). Three loci in Eschrichia coli K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics. **53**, 1119.

Howard-Flanders, P. and Theriot, L. (1962). A method for selecting radiation-sensitive mutants of Escherichia coli. Genetics. **47**, 1219.

Hoy, C. A., Thompson, L. H., Mooney, C. L., and Salazar, E. P. (1985). Defective DNA cross-link removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. Cancer Res. **45**, 1737.

Husain, I., Van Houten, B., Thomas, D. C., and Sancar, A. (1986). Sequences of the uvrA gene and protein reveal two potential ATP binding sites. J. Biol. Chem. **261**, 4895.

Isomura, K., Nikaido, O., Horikawa, M., and Sugahara, T. (1973). Repair of DNA damage in ultraviolet-sensitive cells isolated from HeLa S3 cells. Radiat. Res. **53**, 143.

Jeeves, W. P. and Rainbow, A. J. (1986). An aberration in γ-ray-enhanced reactivation of irradiated adenovirus in ataxia telangiectasia fibroblasts. Carcinogenesis. **7**, 381.

Johnson, D. C., Ghosh-Choudhury, G., Smiley, J. R., Fallis, L, and Graham, F. L. (1988). Abundant expression of herpes simplex virus glycoprotein gB using an adenovirus vector. Virology. **163**, 1.

Kantor, G. J., Barsalou, L. S., and Hanawalt, P. C. (1990). Selective repair of specific chromatin domains in UV-irradiated cells from xeroderma pigmentosum complementation group C. Mutat. Res. **235**, 171.

Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. (1991). Participation of p53 protein in the cellular response to DNA damage. Cancer Res. **51**, 6304.

Kelekar, A., and Cole, M. D. (1987). Immortalization by c-myc, h-ras, and E1A oncogenes induces differential cellular gene expression and growth factor responses. Mol. Cell Biol. **7**, 3899.

Kelner, A. (1949). Effect of visible light on the recovery of *Streptomyces griseus* conidia from ultraviolet irradiation injury. Proc. Natl. Acad. Sci (USA). **35**, 73.

Kenyon, C., and Walker, G. (1980). DNA-damaging agents stimulate gene expression at specific loci in Escherichia coli. Proc. Natl. Acad. Sci. (USA). **77**, 2819.

Kenyon, C. and Walker, G. (1981). Expression of the E. coli uvrA gene is inducible. Nature. **289**, 808.

Kern, S. E., Pietenpol, J. A., Thiagalingam, S., Seymour, A., Kinzler, K. W., and Vogelstein, B. (1992). Oncogenic forms of p53 inhibit p53-regulated gene expression. Science. 256, 827.

Kibitel, J.T., Yee, V., and Yarosh, D.B. (1991). Enhancement of ultraviolet-DNA repair in denV gene transfectants and T4 endonuclease V-liposome recipients. Photochem. Photobiol. **54**, 753.

Kuemmerle, N. B. and Masker, W. E. (1980). Effect of the uvrD mutation on excision repair. J. Bacteriol. **142**, 535.

Kusewitt, D.F., Ley, R.D., and Henderson, E.E. (1991). Enhanced pyrimidine dimer removal in repair-proficient murine fibroblasts transformed with the *denV* gene of bacteriophage T4. Mutat. Res. **255**, 1.

Lane, D. P. (1992). p53, guardian of the genome. Nature, **358**, 15.

Lechner, R. L. and Kelly, T. J. Jr. (1977). The structure of replicating adenovirus 2 DNA molecules. Cell. **12**, 1007.

Ley, R. D., Applegate, L. A., de Riel, J. K., and Henderson, E. E. (1989). Excision repair characteristics of *denV*-transformed xeroderma pigmentosum cells. Mutation Res. **217**, 101.

Lichy, J. H., Field, J. Horwitz, M. S., and Hurwitz, J. (1982). Separation of the adenovirus terminal protein precursor from its associated DNA polymerase: role of both proteins in the initiation of adenovirus DNA replication. Proc. Natl. Acad. Sci. (USA). **79**, 5225.

Lichy, J. H., Horwitz, M. S., and Hurwitz, J. (1981). Formation of a covalent complex between the 80,000 dalton adenovirus terminal protein and 5' dCMP in vitro. Proc. Natl. Acad. Sci. (USA). **78**, 2678.

Lindahl, T. (1979). DNA glycosylases, endonucleases for apurinic/apyrimidinic sites and base excision repair. Prog. Nucleic Acids Res. Mol. Biol. **22**, 135.

Lindahl, T. (1982). DNA repair enzymes. Ann. Rev. Biochem. **51**, 61.

Linne, T. Jonvall, H., and Philipson, L. (1977). Purification and characterization of the phosphorylated DNA binding protein from adenovirus type 2 infected cells. Eur. J. Biochem. **76**, 481.

Little, J. W. and Mount, D. W. (1982). The SOS regulatory system of Escherichia coli. Cell. **29**, 11.

Little, J. W., Mount, D. W., and Yanisch-Perron, C. R. (1981). Purified lexA protein is a repressor of the recA and lexA genes. Proc. Natl. Acad. Sci. (USA). **77**, 4199.

Liuzzi, M., Weinfeld, M., and Paterson, M. C. (1987). Selective inhibition by methoxyamine of the apurinic/apyrimidinic endonuclease activity associated with pyrimidine dimer-DNA glycosylases from *Micrococcus luteus* and bacteriophage T4. Biochemistry **26**, 3315.

Longiaru, M. and Horwitz, M. S. (1981). Chinese hamster ovary cells replicate adenovirus deoxyribonucleic acid. Mol. Cell Biol. **1**, 208.

Luria, S. E. (1947). Reactivation of irradiated bacteriophage by transfer of self-reproducing units. Proc. Natl. Acad. Sci. (USA). **33**, 253.

Lytle, C. D. (1978). Radiation-enhanced virus reactivation in mammalian cells. Natl. Cancer. Inst. Monogr. **50**, 145.

Lytle, C. D., Benane, S. G., and Bockstahler, L. E. (1974). Ultaviolet-enhanced reactivation of *Herpes* virus in human tumour cells. Photochem. Photobiol. **20**, 91.

Lytle, C. D., Day, R. S. III, Hellman, K. B., and Bockstahler, L. E. (1976). Infection of UV-irradiated xeroderma pigmentosum fibroblasts by herpes simplex virus: Study of capacity and Weigle reactivation. Mutat. Res. **36**, 257.

MacInnes, M. A. and Mudgett, J. S. (1990). Cloning of the functional human excision repair gene ERCC-5: Potential gene regulatory features conserved with other human repair genes. In Mutation and the Environment, Part A. pp. 265. Wiley-Liss, Inc.

Madhani, H. D., Bohr, V. A., and Hanawalt, P. C. (1986). Differential DNA repair in transcriptionally active and inactive proto-oncogenes: c-abl and c-mos. Cell. **45**, 417.

Maltzman, W. and Czyzyk, L. (1984). UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. Molec. Cell Biol. **4**, 1689.

Marshall, R. R., Arlett, C. F., Harcourt, S. A., and Broughton, B. A. (1980). Increased sensitivity of cell strains from Cockayne's syndrome to sister chromatid-exchange induction and cell killing by UV light. Mutation Res. **69**, 107.

Massie, B., Gluzman, Y., and Hassell, J. A. (1986). Construction of a helper-free recombinant adenovirus that expresses polyomavirus large T antigen. Mol. Cell Biol. **6**, 2872.

McPartland, A., Green, L., and Echols, H. (1980). Control of recA gene RNA in E. coli: regualtory and signal genes. Cell. **20**, 731.

Mellon, I., Bohr, V. A., Smith, C. A., and Hanawalt, P. C. (1986). Preferential DNA repair of an active gene in human cells. Proc. Natl. Acad. Sci. (USA). **83**, 8878.

Mellon, I., Spivak, G., and Hanawalt, P. C. (1987). Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. Cell. **51**, 241.

Mohrenweiser, H. W., Carrano, A. V., Fertitta, A., Perry, B., Thompson, L. H., Tucker, J. D., and Weber, C. A. (1989). Refined mapping of the three DNA repair genes, ERCC1, ERCC2, and XRCC1, on human chromosome 19. Cytogenet. Cell Genet. **52**, 11.

Moolenaar, G. F., van Sluis, C. A., Backendorf, C., and van de Putte, P. (1987). Regulation of the Escherichia coli excision repair gene uvrC. Overlap between the uvrC structural gene and the region coding for a 24 kD protein. Nucleic Acids Res. **15**, 4273.

Morin, J. E., Lubeck, M. D., Barton, J. E., Conley, A. J., Davis, A. R., and Hung, P. P. (1987). Recombinant adenoviruses induce antibody response to hepatitis B virus surface antigen in hamsters. Proc. Natl. Acad. Sci. (USA). **84**, 4626.

Mount, D. W., Low, K. B., and Edmiston, S. J. (1972). Dominant mutations (lex) in Escherichia coli K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. **112**, 886.

Mullenders, L. H. F., Venema, J., van Hoffen, A., Sakkers, R. J., Natarajan, A. T., and van Zeeland, A. A. (1991). Genomic heterogeneity of UV-induced repair: relationship to higher-order chromatin structure and transcriptional activity. In Radiation Research: A Twentieth-Century Perspective. Volume II Congress Proceedings (eds. W. C. Dewey, M. Edington, R. J. M. Fry, E. J. Hall, and G. F. Whitmore) pp. 155. Academic Press, San Diego.

Nairn, R. S., Humphrey, R. M., and Adair, G. M. (1988). Transformation of UV-hypersensitive Chinese hamster ovary cell mutants with UV-irradiated plasmids. Int. J. Radiat. Biol. **53**, 249.

Nakabeppu, Y., Yamashita, K., and Sekiguchi, M. (1982). Purification and characterization of normal and mutant forms of T4 endonuclease V. J. Biol. Chem. **257**, 2556.

Nickell, C. and Lloyd, R. S. (1991). Mutations in endonuclease V that affect both protein-protein association and target site location. Biochemistry. **30**, 8638.

Norrby, E., Bartha, A., Boulanger, P., et al. (1976). Adenoviridae. Intervirology. **7**, 117.

Oh, E. Y., Classen, S., Thiagalingam, S., Mazur, S., and Grossman, L. (1989). ATPase activity of the UvrA and UvrB protein complexes of the E. coli UvrABC endonuclease. Nucl. Acids Res. **17**, 4145.

Oh, E. Y. and Grossman, L. (1989). Characterization of the helicase activity of the E. coli UvrAB-protein complex. J. Biol. Chem. **264**, 1336.

Orren, D. K. and Sancar, A. (1989). Subunits of ABC excinuclease interact in solution in the absence of DNA. UCLA Symp. Mol. Cell Biol. **83**, 87.

Paabo, S. T., Nilsson, T., and Peterson, P. A. (1986). Adenoviruses of subgenera B, C, D, and E modulate cell-surface expression of major histocompatibility complex class I antigens. Proc. Natl. Acad. Sci. (USA). **83**, 9665.

Pearson, G. D. and Hanawalt, P. C. (1971). Isolation of DNA replication complexes from uninfected and adenovirus-infected HeLa cells. J. Mol. Biol. **62**, 65.

Persson, H., Kvist, S., Ostberg, L., Petersson, P. A., and Philipson, L. (1979). Adenovirus early glycoprotein E3-19K and its association with transplantation antigens. Cold Spring Harbor Symp. Quant. Biol. **44**, 509.

Prevec, L., Campbell, J. B., Christie, B. S., Belbeck, L., and Graham, F. L. (1990). A recombinant human adenovirus vaccine against rabies. J. Infect. Disease. **161**, 27.

Radna, R. L., Foellmer, B., Feldman, L. A., Francke, U., and Ozer, H. L. (1987). Restriction of human adenovirus replication in Chinese hamster cell lines and their hybrids with human cells. Virus Research. **8**, 277.

Rainbow, A. J. (1977). UV-induced alkaline labile DNA damage in human adenovirus and its repair after infection of human cells. Photochem. Photobiol. **25**, 457.

Rainbow, A. J. (1980). Reduced capacity to repair irradiated adenovirus in fibroblasts from xeroderma pigmentosum heterozygotes. Cancer Res. **40**, 3945.

Rainbow, A. J. (1981). Reactivation of viruses. In Stitch, H. F. and San, R. H. C. (eds.) Short-Term Tests for Chemical Carcinogens. Springer-Verlag, New York. pp. 25.

Rainbow, A. J. (1989a). Relative repair of adenovirus damaged by sunlamp, UV and γ-irradiation in Cockayne syndrome fibroblasts is different from that in xeroderma pigmentosum fibroblasts. Photochem. Photobiol. **50**, 201.

Rainbow, A. J. (1989b). Thymidine kinase deficient human cells have increased UV sensitivity in their capacity to support herpes simplex virus but normal UV sensitivity for colony formation. Mutat. Res. **218**, 189.

Rainbow, A. J. (1989c). Defective repair of UV-damaged DNA in human tumor and SV40-transformed human cells but not in adenovirus-transformed human cells. Carcinogenesis. **10**, 1073.

Rainbow, A. J. (1991). Host cell reactivation of sunlamp-exposed adenovirus in fibroblasts from patients with Bloom's syndrome, ataxia telangiectasia, and Huntington's disease. Envnt. Mol. Mutagenesis. **17**, 98.

Rainbow, A. J. and Howes, M. (1980). Reduced DNA repair in Huntington's Chorea. Radiat. Res. **83**, 475. (Abstract).

Rainbow, A. J. and Howes, M. (1982). A deficiency in the repair of UV and γ-ray damaged DNA in fibroblasts from Cockayne's syndrome. Mutat. Res. **93**, 235.

Rainbow, A. J. and Castillo, J. E. (1992). Adenovirus as a molecular probe to study homologous recombination in mammalian cells: enhanced recombination following UV-irradiation of the virus. Mutat. Res. (in press).

Regan, J. D., Thompson, L. H., Carrier, W. L., Weber, C. A., Francis, A. A., and Zdzienicka, M. Z. (1990). Cyclobutane-pyrimidine dimer excision in UV-sensitive CHO mutants and the effect of the human ERCC2 repair gene. Mutat. Res. **235**, 157.

Rekosh, D. M. K., Russell, W. C., Bellet, A. J. D., and Robinson, A. J. (1977). Identification of a protein linked to the ends of adenovirus DNA. Cell. **11**, 283.

Robbins, J. H., Kraemer, K. H., Lutzner, M. A., Festoff, B. W., and Coon, H. G. (1974). Xeroderma pigmentosum. An inherited disease with sun sensitivity, multiple cutaneous neoplasms and abnormal DNA repair. Ann. Int. Med. **80**, 221.

Rubin, J. S. and Whitmore, G. F. (1985). DNA repair-deficient Chinese hamster ovary cells exhibiting differential sensitivity to γ rays under aerobic and hypoxic conditions. Radiation Res. **101**, 528.

Ruley, J. E. (1983). Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature. **304**, 602.

Rowe, D. T., Branton, P. E., and Graham, F. L. (1984). The kinetics of synthesis of early viral proteins in KB cells infected with wild-type and transformation defective host-range mutants of human adenovirus type 5. J. Gen. Virol. **65**, 585.

Rowe, W. P., Huebner, R. J., Gillmore, L. K., Parrott, R. H., and Ward, T. G. (1953). Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exp. Biol. Med. **84**, 570.

Rupert, C. S. (1975). Enzymatic photoreactivation of DNA. In DNA repair mechanisms. P. C. Hanawalt, E. C. Friedberg, C. F. Fox, eds., p. 113. New York: Academic.

Rupert, C. and Harm, W. (1966). Reactivation after photobiological damage. Adv. Radiat. Biol. **2**, 1.

Rupp, W. D. and Howard-Flanders, P. (1968). Discontinuities in the DNA synthesized in an excision-defective strain of Escherichia coli following ultraviolet irradiation. J. Mol. Biol. **31**, 291.

Rupprecht, C. E., Wiktor, T. J., Johnston, D. H., Haimer, A. N., Dietzschold, B., Wunner, W. H., Glickman, L. T., and Koprowski, H. (1986). Oral immunization and protection of raccoons (*Procyon lotor*) with a vaccinia-rabies glycoprotein recombinant virus vaccine. Proc. Natl. Acad. Sci. (USA). **83**, 7947.

Ryan, D. K. G. and Rainbow, A. J. (1986). Comparative studies of host-cell reactivation, cellular capacity and enhanced reactivation of herpes simplex virus in normal, xeroderma pigmentosum and Cockayne syndrome fibroblasts. Mutat. Res. **166**, 99.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular cloning: a laboratory manual, second edition. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York.

Sancar, A., Franklin, K. A., and Sancar, G. B. (1984). Escherichia coli DNA photolyase stimulates UvrABC excision nuclease in vitro. Proc. Natl. Acad. Sci. (USA). **81**, 7397.

Sancar, G. B., Sancar, A., Little, J. W., and Rupp, W. D. (1982). The *uvrB* gene of Escherichia coli has both lexA-repressed and lexA-independent promoters. Cell. **28**, 523.

Sancar, G. B., Sancar, A., and Rupp, W. D. (1984). Sequences of the Excherichia coli *uvrC* gene and protein. Nucleic Acids Res. **12**, 4593.

Sarnow, P., Hearing, P., Anderson, C. W., Reich, N., and Levine, A. J. (1982). Identification and characterization of an immunologically conserved adenovirus early region 11,000 M_r protein and its association with the nuclear matrix. J. Mol. Biol. **162**, 565.

Scharer, E. and Iggo, R. (1992). Mammalian p53 can function as a transcription factor in yeast. Nucleic Acids Res. **20**, 1539.

Schneider, M., Graham, F. L., and Prevec, L. (1989). Expression of the glycoprotein of vesicular stomatitis virus by infectious adenovirus vectors. J. gen. Virol. **70**, 417.

Setlow, R. B. (1978). Repair deficient human disorders and cancer. Nature. **271**, 713.

Setlow, R. B., Swenson, P. A., and Carrier, W. L. (1963). Thymine dimers and inhibition of DNA synthesis by ultraviolet irradiation of cells. Science. **142**, 1464.

Shiomi, T. and Sato, K. (1979). Isolation of UV-sensitive variants of human FL cells by a viral suicide method. Somat. Cell Genet. **5**, 193.

Shivji, M. K. K., Kenny, M. K., and Wood, R. D. (1992). Proliferating cell nuclear antigen is required for DNA excision repair. Cell. **69**, 367.

Smith, C. A. and Hanawalt, P. C. (1978). Phage T4 endonuclease V stimulates DNA repair replication in isolated nuclei from ultraviolet-irradiated human cells, including xeroderma pigmentosum fibroblasts. Proc. Natl. Acad. Sci. (USA). **75**, 2598.

Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**, 503.

Squires, S., Johnson, R. T., and Collins, A. R. S. (1982). Initial rates of DNA incision in UV-irradiated human cells. Differences between normal, xeroderma pigmentosum and tumor cells. Mutat. Res. **95**, 389.

Stefanini, M., Collins, A. R., Riboni, R., Klaude, M., Botta, E., Mitchell, D. L., and Nuzzo, F. (1991). Novel Chinese hamster UV-sensitive mutants for excision repair form complementation groups 9 and 10. Cancer Research. **51**, 3965.

Stefanini, M., Mondello, C., Botta, E., Riboni, R., and Nuzzo, F. (1989). Cellular and genetic characterization of UV sensitive Chinese hamster mutants. Ann. 1st Super. Sanita. **25**, 123.

Stefanini, M., Reuser, A., and Bootsma, D. (1982). Isolation of Chinese hamster ovary cells with reduced unscheduled DNA synthesis after UV irradiation. Somatic Cell Genet. **8**, 635.

Sutherland, B. M. (1974). Photoreactivating enzyme from human leukocytes. Nature. **248**, 109.

Sutherland, B. M., Runge, P., and Sutherland, J. C. (1974). DNA photoreactivating enzyme from placental mammals. Origin and characterization. Biochemistry. **13**, 4710.

Sutherland, J. C. and Griffin, K. P. (1980). Monomerization of pyrimidine dimers in DNA by tryptophan-containing peptides: wavelength dependence. Radiat. Res. **83**, 529.

Tanaka, K., Miura, N., Satokata, I., Miyamoto, I., Yoshida, M. C., Satoh, Y., Kondo, S., Yasui, A., Okayama, H., and Okada, Y. (1990). Analysis of human DNA excision repair gene involved in group A xeroderma pigmentosum and containing a zinc-finger domain. Nature. **348**, 73.

Tanaka, K., Satokata, I., Ogita, Z., Uchida, T., and Okada, Y. (1989). Molecular cloning of a mouse DNA repair gene that complements the defect of group-A xeroderma pigmentosum. Proc. Natl. Acad. Sci. (USA). **86**, 5512.

Tang, K. M. (1991). Construction of a herpes simplex virus type 1 (HSV-1) expression vector containing the bacteriophage T4 *denV* gene: Effect of this gene on UV-survival of HSV-1 in normal and xeroderma pigmentosum fibroblasts. M. Sc. thesis. McMaster University, Hamiltion, Ontario.

Taylor, A. M. R., Harnden, D. G., Arlett, C. F., Harcourt, S. A., Lehmann, A. R., Stevens, S., and Bridges, B. A. (1975). Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. Nature. **258**, 427.

Terleth, C., van de Putte, P., and Brouwer, J. (1991). New insights in DNA repair: preferential repair of transcriptionally active DNA. Mutagenesis. **6**, 103.

Thompson, L. H., Busch, D. B., Brookman, K., Mooney, C. L., and Glaser, D. A. (1981). Genetic diversity of UV-sensitive DNA repair mutants of Chinese hamster ovary cells. Proc. Natl. Acad. Sci. (USA). **78**, 3734.

Thompson, L. H., Brookman, K. W., Dillehay, L. E., Carrano, A. V., Mazrimas, J. A., Mooney, C. A., and Minkler, J. L. (1982). A CHO-cell strain having hypersensitivity to mutagens, a defect in strand-break repair, and an extraordinary baseline frequency of sister chromatid exchange. Mutat. Res. **95**, 247.

Thompson, L. H., Brookman, K. W., Dillehay, L. E., Mooney, C. L., and Carrano, A. V. (1982). Hypersensitivity to mutation and sister-chromatid-exchange induction in CHO cell mutants defective in incising DNA containing UV lesions. Somatic Cell Genet. **8**, 759.

Thompson, L. H. and Carrano, A. V. (1983). In Cellular Responses to DNA Damage. UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 11 (eds. E. C. Friedberg and B. A. Bridges). pp.125 Alan R. Liss, New York.

Thompson, L. H., Fong, S., and Brookman, K. (1979). Validation of conditions for efficient detection of *hprt* and *aprt* mutations in suspension-cultured Chinese hamster ovary cells. Mutat. Res. **74**, 21.

Thompson, L. H., Mitchell, D. L., Regan, J. D., Bouffler, S. D., Stewart, S. A., Carrier, W. L., Nairn, R. S., and Johnson, R. T. (1988). CHO mutant UV61 removes (6-4) photoproducts but not cyclobutane dimers. Mutagenesis. **4**, 140.

Thompson, L. H., Mitchell, D. L., Regan, J. D., Bouffler, S. D., Stewart, S. A., Carrier, W. L., Nairn, R. S., and Johnson, R. T. (1989). CHO mutant UV61 removes (6-4) photoproducts but not cyclobutane dimers. Mutagenesis. **4**, 140.

Thompson, L. H., Rubin, J. S., Cleaver, J. E., Whitmore, G. F., and Brookman, K. (1980). A screening method for isolating DNA repair-deficient mutants of CHO cells. Somatic Cell Genet. **6**, 391.

Thompson, L. H., Salazar, E. P., Brookman, K. W., Collins, C. C., Stewart, S. A., Busch, D. B., and Weber, C. A. (1987). Recent progress with the DNA repair mutants of Chinese hamster ovary cells. In Molecular Biology of DNA Repair (eds. A. Collins, R. T. Johnson, and J. M. Boyle). pp.97 (The Company of Biologists Limited, Cambridge). J. Cell Sci. Suppl. **6**, 97.

Thompson, L. H., Shiomi, T., Salazar, E. P., and Stewart, S. A. (1988). An eighth complementation group of rodent cells hypersensitive to ultraviolet radiation. Som. Cell Mol. Genet. **14**, 605.

Todd, P., Coohill, T. P., and Mahoney, J. A. (1968). Responses of cultured Chinese hamster cells to ultraviolet light of different wavelengths. Radiat. Res. **35**, 390.

Troelstra, C., Landsvater, R. M., Wiegant, J., van der Ploeg, M., Viel, G., Buys, C. H. C. M., and Hoeijmakers, J. H. J. (1992). Localization of the nucleotide excision repair gene ERCC6 to human chromosome 10q11-q21. Genomics. **12**, 745.

Troelstra, C., Odijk, H., de Wit, J., Westerveld, A., Thompson, L. H., Bootsma, D., and Hoeijmakers, J. H. J. (1990). Molecular cloning of the human DNA excision repair gene ERCC-6. Mol. Cell Biol. **10**, 5806.

Valerie, K., de Riel, J. K., and Henderson, E. E. (1985). Genetic complementation of UV-induced DNA repair in Chinese hamster ovary cells by the *denV* gene of phage T4. Proc. Natl. Acad. Sci. (USA). **82**, 7656.

Valerie, K., Green, A. P., de Riel, J. K., and Henderson, E. E. (1987). Transient and stable complementation of ultraviolet repair in xeroderma pigmentosum cells by the *denV* gene of bacteriophage T4. Cancer Res. **47**, 2967.

Valerie, K., Henderson, E. E., and de Riel, J. K. (1984). Identification, physical map location and sequence of the *denV* gene from bacteriophage T4. Nucleic Acids Res. **12**, 8085.

van den Berg, J., Jaspers, N. G. J., Odijk, J. H. M., van der Spek, P., Koken, M. H. M, van Duin, M., Hoeijmakers, J. H. J., and Bootsma, D. (1991). (Abstract) Functional analysis of the ERCC-1 gene and protein.

van der Eb, A. J., van den Elsen, P., Jochemsen, H., et al (1979). Structure and function of adenovirus 5 transformation genes. J. Supramol. Struct. [Suppl]. **3**, 128.

van der Vliet, P. C. and Sussenbach, J. S. (1972). The mechanism of adenovirus DNA synthesis in isolated nuclei. Eur. J. Biochem. **30**, 584.

van Duin, M., de Wit, J., Odijk, H., Westerveld, A., Yasui, A., Koken, M. H. M., Hoeijmakers, J. H. J., and Bootsma, D. (1986). Molecular characterization of the human excision repair gene ERCC-1: cDNA cloning and amino acid homology with the yeast DNA repair gene RAD10. Cell. **44**, 913.

van Duin, M., Janssen, J. H., de Wit, J., Hoeijmakers, J. H. J., Thompson, L. H., Bootsma, D., and Westerveld, A. (1988). Transfection of the cloned human excision repair gene *ERCC-1* to UV-sensitive CHO mutants only corrects the repair defect in complementation group-2 mutants. Mutat. Res. **193**, 123.

van Duin, M., van den Tol, J., Warmerdam, P., Odijk, H., Meijer, D., Westerveld, A., Bootsma, D., and Hoeijmakers, J. H. J. (1988). Evolution and mutagenesis of the mammalian excision repair gene *ERCC-1*. Nucleic Acids Res. **16**, 5305.

Venema, J., Mullenders, L. H. F., Natarajan, A. T., van Zeeland, A. A., and Mayne, L. V. (1990). The genetic defect in Cockayne's syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. Proc. Natl. Acad. Sci. (USA). **87**, 4707.

Venema, J., van Hoffen, A., Natarajan, A. T., van Zeeland, A. A., and Mullenders, L. H. F. (1990). The residual repair capacity of xeroderma pigmentosum complementation group C fibroblasts is highly specific for transcriptionally active DNA. Nucleic Acids Res. **18**, 443.

Weber, C. A., Salazar, E. P., Stewart, S. A., and Thompson, L. H. (1988). Molecular cloning and biological characterization of a human gene, *ERCC2*, that corrects the nucleotide excision repair defect in CHO UV5 cells. Mol. Cell Biol. **8**, 1137.

Weber, C. A., Salazar, E. P., Stewart, S. A., and Thompson, L. H. (1990). *ERCC2*: cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast RAD3. EMBO. **9**, 1437.

Weber, C. A. , Thompson, L. H., and Salazar, E. P. (1991). Characterization of *ERCC2*, a human nucleotide excision repair gene that corrects XP group D. Ninth International Congress of Radiation Research, Toronto, Ontario.

Weeda, G., van Ham, R. C. A., Vermeulen, W., Bootsma, D., van der Eb, A. J., and Hoeijmakers, J. H. J. (1990). A presumed DNA helicase encoded by *ERCC-3* is involved in the human repair disorders xeroderma pigmentosum and Cockayne's syndrome. Cell. **62**, 777.

Weigle, J. J. (1953). Induction of mutations in a bacterial virus. Proc. Natl. Acad. Sci. (USA). **39**, 628.

Weiss, B., and Grossman L. (1987). Phosphodiesterases involved in DNA repair. In Advances in Enzymology (ed. A. Meister) pp. 1.

Westerveld, A., Hoeijmakers, J. H. J., van Duin, M., de Wit, J., Odijk, H., Pastink, A., Wood, R. D., and Bootsma. (1984). Molecular cloning of a human DNA repair gene. Nature. **310**, 425.

Wolfson, J. and Dressler, D. (1972). Adenovirus DNA contains an inverted terminal repetition. Proc. Natl. Acad. Sci. (USA). **69**, 3054.

Yamaizumi, M., Inaoka, T., Uchida, T., and Ohtsuka, E. (1989). Microinjection of T4 endonuclease V produced by a synthetic *denV* gene stimulates

unscheduled DNA synthesis in both xeroderma pigmentosum and normal cells. Mutat. Res. **217**, 135.

Zdzienicka, M. Z., van der Schans, G. P., and Simons, J. W. I. M. (1988). Identification of a new seventh complementation group of UV-sensitive mutants in Chinese hamster ovary cells. Mutat. Res. **194**, 165.

Zelle, B. and Lohman, P. H. M. (1979). Repair of UV-endonuclease-susceptible sites in the 7 complementation groups of xeroderma pigmentosum A through G. Mutat. Res. **62**, 363.