UV ENHANCED MUTAGENESIS OF ADENOVIRUS

IN HUMAN FIBROBLASTS

by

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ABSTRACT

UV-induced cellular "error-prone" repair was examined by the comparative determination of the enhanced reactivation (UVER) and mutagenesis (UVEM) among viral progeny resulting from single cycle lytic infections of intact or UV-irradiated Ad5ts36 and Ad5ts125 in unirradiated or UV-irradiated confluent human fibroblasts. The induction of phenotypic wild type revertants among the viral progeny was determined by plaquing at 33°C (permissive) and 39°C (nonpermissive) on HeLa or KB indicator monolayers. UV-irradiation of Ad5ts36 and Ad5ts125 resulted in a dose dependent increase in the UV-induced reversion frequency (RF) of viral progeny and a dose dependent exponential decrease in progeny survival in unirradiated normal fibroblasts. Kinetic analysis of UV-induced reversion suggests that 2.5 ± 0.3 (Ad5ts36) and 2.4 ± 0.5 (Ad5ts125) "hits" were required to produce a targeted reversion event among the viral progeny derived from normal human fibrolasts. The expression of UVER and UVEM was examined as a function of increasing time delay between cellular UV-irradiation and viral infection for various time periods. UV-irradiation of normal human fibroblasts (10 J/m/2●), carried out 24 hours prior to viral infection, resulted in an increased RF for both unirradiated (untargeted increase) or UV-irradiated (targeted increase) virus. A maximal UVEM of 1.4 ± 0.2 was observed concomitantly with the maximal UVER (3.4 ± 0.8) and targeted increase (1.9 ± 0.3) when viral infection was delayed 24 hours following cellular UV-irradiation.

The maximal untargeted increase (2.0 ± 0.5) was observed when viral infection was immediately following cellular irradiation and declined as viral infections were delayed for longer time intervals.

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Different time courses for the targeted and untargeted increases suggested that these·processes may be separately regulated in human fibroblast cells.

DNA repair-deficient human fibroblast strains from patients with ataxia telangiectasia (AT), xeroderma pigmentosum (XP) and Cockyane's syndrome (CS) were also examined for the expression of UVER and/or UVEM of UV-irradiated adenovirus. AT fibroblasts expressed a significantly reduced UVEM (0.31 ± 0.12) of UV-irradiated adenovirus as compared to normals when viral infections were delayed 24 hours following cellular UV-irradiation. AT fibroblasts also displayed abnormally reduced UVER of UV-irradiated adenovirus when viral infections ·immediately followed cellular UV-irradiation. Aberrant expression of both UVER and UVEM of UV-irradiated ·adenovirus in AT suggests that these cells may be deficient in an error-prone DNA repair process or express enhanced error-free repair. Similarly, the XP variant strain studied displayed some aberration of UVEM when compared to normals indicated by a reduced UVEM (0.52 ± 0.2) of UV-irradiated adenovirus for viral infections delayed 24 hours after cellular irradiation. UV-induced reversion of adenovirus progeny derived from unirradiated XP25RO (complementation group A) was hypermutable on a per unit dose basis but when corrected for survival, reversion was similar to that, seen for normals. This suggests excision repair is an error-free. process. UVER and UVEM of UV-irradiated adenovirus in CS fibroblasts (a non cancer prone ·condition) were similar to that observed in normals when viral infections were 24 hours after cellular irradiation. These results suggest that fibroblasts from cancer-prone individuals (AT, XPA, XP var) all express abnormal ·parameters of

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UV-induced mutagenesis. The underlying mechanism(s) responsible for this abnormal mutagenesis may ultimately contribute to the cancer-prone nature of these syndromes.

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INTRODUCTION

A large amount of experimental data has accumulated in the field of DNA damage and repair utilizing the new methods associated with the expanding field of molecular biology. UV-irradiation has been the DNA damaging agent of choice for many studies due to its availability, ease of use, and safety, while expressing the principal biological effects of lethality and mutagenesis. Cells have evolved both constitutive and inducible enzymatic repair systems which can correct or bypass the lethal DNA photoproducts associated with UV-irradiation of DNA in biological systems. The accuracy (or fidelity) of the subsequent DNA repair or replicative bypass can be either high (error-free) or low (error-prone). If the repair has low fidelity then mismatched deoxynucleoside monophosphates will be inserted into the DNA and if these "lesions" are tolerated beyond DNA replication, one of the daughter strands will pass an altered (mutant) genome to succeeding generations. The misincorporated nucleotides undergo a "fixation" process during DNA replication following UV-repair. The resulting mutational changes may be either silent (in a region of DNA that is noncoding, or a codon change which will code for the same amino acid) or be expressed as an altered biologic phenotype due to changes in protein amino acid sequence. Error-prone repair/recovery mechanisms have been suggested as a possible mechanism involved in the production of abnormally proliferative transformed cells. Proponents of the somatic mutation theory of carcinogenesis frequently cite the relationship that human mutants deficient in

excision repair of UV-damaged DNA have elevated levels of cancer (Bridges, 1981; Straus, 1981). Patients with the autosomal recessive syndrome Xeroderma pigmentosum (XP) develop multiple tumors of the skin exposed to UV light (Robbins et al, 1974; Kraemer, 1977; Setlow, 1978). Similarly, the hereditary chromosome instability syndromes Ataxia telangiectasia, Fanconi's anaemia and Bloom's syndrome have a high risk for the development of malignant neoplasias and have all been associated with abnormal DNA repair (Paterson and Smith, 1979; Bridges 1981; Straus, 1981).

Recent studies examining the molecular mechanism associated with the T24 and EJ bladder carcinoma phenotype have revealed that the activation of the cellular Ha-ras 1 oncogene by a point mutation results in a single amino acid change that is sufficient to fully transform the mouse NIH 3T3 cell line. These transforming oncogenes presumably arise from normal cellular genes by mutation (Goldfarb et al, 1982; Shih and Weinberg, 1982; Tabin et al, 1982; Reddy et al, 1982). A somatic mutational event possibly mediated by error-prone repair has been suggested as a mechanism of cancer since rat mammary carcinomas resulting from a single dose of the potent alkylating agent nitroso-methylurea induced the activation of the H-ras-1 oncogene by a targeted mutational event at the 12th codon (Sukumar et al, 1983). This implies that a tumor may arise from the somatic activation of a normal cellular oncogene, possibly through an error-prone repair of targeted DNA damage. In light of the above correlations it is of great interest to identify the mechanisms of both prokaryotic and eukaryotic error-prone repair processes in order to elucidate its role in

mammalian carcinogenesis. Following is a brief review of DNA repair and mutagenesis in bacterial and mammalian systems with emphasis on the use of UV light as a DNA damaging agent.

I. Bacterial UV-Repair

UV-light, in the range of 240 to 300 nm is efficiently absorbed by DNA and produces cyclobutane pyrimidine dimers between adjacent pyrimidines such that the alpha-helical structure of the DNA is distorted. Other photoproducts of UV-irradiation are formed but lethality and mutagenesis have been attributed to the cyclobutane pyrimidine dimers for the following reasons: they are the most prevalent lesion, enzymatic photoreversal of the dimers by visible light reverses the lethal effects, and decreases the mutational activity of UV, and bacterial mutants deficient in repair of cyclobutane dimers show greatly increased sensitivity to the lethal and mutagenic effects of UV (Haseltine, 1983). Two major classes of UV-sensitive mutants have been identified in E. coli. Mutants have been found that are deficient in excision repair (uvrA⁻), deficient in replication of UV-damaged DNA (recA⁻), or are deficient in both (uvrA⁻ recA⁻) (Hall and Mount, 1981). A mutant deficient in both processes can be killed in the presence of one or two pyrimidine dimers, suggesting that one unrepaired dimer can be lethal (Hall and Mount, 1981). Following is a brief summary of the constitutive and inducible DNA repair systems identified in prokaryotes.

Excision Repair of UV-damaged DNA

In E. coli UV-damaged regions of DNA can be repaired via an

enzyme mediated nucleotide excision event which is followed by the resynthesis of DNA in the damaged region called gap filling (Boyce and Howard-Flanders, 1964; Setlow and Carrier, 1964; Howard-Flanders, et. al. 1966; Witkin, 1976). The postulated excision-repair pathway has been based on biochemical, genetic, and enzymatic studies including the analysis of two UV-specific endonucleases, one purified from M. luteus and the other encoded by phage T_4 (Haseltine, 1983). The mechanism of excision repair has been found to be multienzymatic and error-free (Witkin, 1976; Hanawalt et al, 1979; Seeberg et al, 1980). In E. coli three proteins (UvrA, UvrB and UvrC) have been implicated in excision repair since mutants defective at any of these genetic loci become extremely sensitive to UV damage in vivo (Rupp and Howard-Flanders, 1968; Shimada et al, 1968; Seeberg et al, 1980) and in vitro (Seeberg et al, 1976). By using plasmids which greatly over-produce these three proteins, milligram quantities of the UvrABC proteins were reconstituted in vitro (Sancar and Rupp, 1983). The reconstituted enzyme made two cuts at the UV-damaged site, one on each side of the pyrimidine dimer such that a 12-13 nucleotide long fragment dissociates from the helix (Sancar and Rupp, 1983). The excision of the pyrimidine dimers is followed by repair synthesis by DNA polymerase 1 which fills the excision gaps and is then sealed by DNA ligase (Witkin, 1976; Hanawalt, et al, 1979; Seeberg et al, 1980; Hanawalt, 1983; Sancar and Rupp, 1983). Repair patches have been found to be heterogeneous in size (Cooper and Hanawalt, 1972; Berstein, 1981) and the production of long patches but not short patches requires the presence of a functional recA and lex A genotype which has also been found essential

for "SOS" error-prone repair (Witkin, 1976; Cooper, 1982).

A ligation event involving DNA polynucleotide ligase functions to restore the primary structure of the DNA (Youngs and Smith, 1977).

In E. coli, M. luteus, and B. subtilus, excision repair has been found to be constitutively expressed (Witkin, 1976; Kimball, 1978; Yasbin et al, 1979; Fields and Yasbin, 1983). Under "SOS" inducing conditions, excision repair is expressed to greater levels through depression of the SOS regulon (Little and Mount, 1982; Walker, 1984). Thus, excision repair is both constitutive and inducible depending on the cells physiological state.

Base Excision Repair

Repair of UV-damaged DNA may be mediated by a large group of N-glycosylase enzymes that specifically recognize modified bases, hydrolyze the N-glycosylic bond to which they are attached, liberate a free modified base and generate an apurinic (AP) or apyrimidinic (Apy) site (Grossman, 1981; Lindahl, 1982). Repair mediated by these enzymes has been termed base excision-repair. These enzymes have been identified in bacterial and mammalian cells; they usually exhibit low molecular weight and are highly damage specific, recognizing a very limited number of base modifications and do not correct mismatched bases or remove 5-methylcytosine (Lindahl, 1982; Haseltine, 1983). The AP or Apy site can be rapidly corrected by an excision-repair process that involves the replacement of a single nucleotide residue or at most a small number of nucleotides (Lindahl, 1982).

Post-Replication Repair

The treatment of bacteria with UV light results in a dose dependent inhibition of DNA synthesis which can be reversed using photoreactivating enzyme suggesting that pyrimidine dimers are responsible for DNA blockage (Witkin, 1976; Hall and Mount, 1981). Once replication is blocked, division of the UV-irradiated E. coli is inhibited by either one of two pathways which depend on activation of "SOS" repair controlled by the lex A and recA gene products (see below) (Burton and Holland, 1983). The extent of DNA replication inhibition is greater per unit dose in excision-deficient strains, which suggests normal rates of DNA replication can resume after excision of pyrimidine dimers (Witkin, 1976; Hall and Mount, 1981). UV-induced lesions encountered by actively replicating DNA will block newly synthesized DNA and reinitiate synthesis after the lesion leaving a SS gapped duplex (Rupp and Howard-Flanders, 1968; Witkin, 1976; Hall and Mount, 1981). Pulse-labelled DNA from UV-irradiated cells will initially appear as short (low molecular weight) strands, which eventually become associated with longer DNA after cells are chased in unlabelled medium (Rupp and Howard-Flanders, 1968). The elongation process eliminates the gaps and is generally referred to as post-replication repair. This process allows cells to replicate DNA in the presence of UV-damage.

Two mechanisms of post-replication repair of gaps have been postulated. One mechanism involves the relaxation of fidelity in the DNA replication process such that a bypass or transdimer synthesis can occur (Witkin, 1976; Kimball, 1978; Hall and Mount, 1981; Rabkin et al, 1983). The other mechanism involves gap-filling in nascant strands by

recombinational strand exchange with a sister chromosome. Recombinational post replication repair requires excision repair to fill the resulting gaps in the donor parental strand and also requires the multifunctional recA gene product to mediate the strand exchanges (Smith and Meun, 1970; Rupp et al, 1971). The recA protein of E. coli is a specific protease and a DNA dependent ATPase that promotes homologous pairing of a partially SS DNA molecule and an intact duplex molecule of DNA (Cunningham et al, 1980; Gottesman, 1981; Radding, 1981, Dressler and Potter, 1982).

The SOS Regulatory System

The SOS regulatory system mediates the response of E.coli. to a variety of treatments (including UV) that damage DNA such that the expression of these functions are believed to aid in the cells survival (Radman, 1975; Witkin, 1976; Little and Mount, 1982; Kenyon, 1983; Little, 1983). Any inhibition in DNA replication (such as that resulting from DNA damage) will activate the following functions in E. coli: enhanced DNA repair (both excision and post replication repair), Weigle reactivation, Weigle mutagenesis, enhanced cellular mutagenesis, inhibition of cell division, prophage induction, cessation of cellular respiration, alleviation of host controlled restriction and induction of stable DNA replication (Little and Mount, 1982). The system depends on the regulation of two proteins such that it can exist in either of two states: in the repressed state, the LexA repressor is active and it represses the set of unlinked "SOS" genes; in the induced state, the RecA protein acquires a protease function which cleaves and inactivates

the LexA repressor resulting in derepression of the "SOS" regulon with enhanced expression of the above "SOS" functions (Little, 1983).

The LexA protein represses the RecA protease such that when the SOS system is turned "off" all the target genes (including rec A) only display a low level of expression (Little and Mount, 1982). When DNA damage has occurred, an inducing signal (of unknown identity) activates the RecA protease and LexA repressors are cleaved, thus derepressing the SOS regulon which participates in the repair of the damaged DNA (Little, 1983). Both the excision repair genes uvr A and uvr B are under lex A repressor inhibition as well as the umu C gene which has been associated with increased mutagenesis (Kenyon and Walker, 1981; Fogliano and Schendel, 1981; Little and Mount, 1982). Inducible UV-mutagenesis requires the activation of the umu C gene (Kato and Shinoura, 1977). This has been suggested since rec A lex A umu C⁻ cells have been found to be UV non mutable (Painter, 1983). Rec A lex A, and umu C all belong to a class of about 10 unlinked genes called din (damage-inducible) (Sarasin, 1982; Little and Mount, 1982).

The kinetics of induction and decay of the SOS error-prone repair system has suggested that the repair system is inducible (Witkin, 1976; Hanawalt et al, 1979; Gottesman, 1981; Little and Mount, 1982). A further indication of the inducibility of the SOS regulon is the observation that protein synthesis inhibitors will prevent UV induction of the SOS responses (Witkin, 1976; Bridges and Mottershead, 1978; Kimball, 1978). UV-mutagenesis in E. coli treated with chloramphenicol has suggested that there may be a constitutive error-prone repair of lesions associated with preexisting DNA since the

frequency of UV-induced mutations was greatly reduced by chloramphenicol pretreatment (Bridges and Mottershead, 1978). The latter effect of chloramphenicol has been attributed to the need of an active replication fork for UV-induced mutagenesis.

Weigle Reactivation, Weigle Mutagenesis and Error-Prone Repair of Bacteriophage

The use of bacteriophage probes of bacterial DNA repair have demonstrated the inducible nature of the SOS regulon's expression. The kinetics of induction of Weigle-reactivation (WR) for UV-irradiated phage suggested this was representative of an inducible SOS function with maximal reactivation seen 30 minutes after UV-irradiation of the host cells (Defais et al, 1971). The process of WR is defined as the increase in survival of UV-irradiated phage in host cells that have been preirradiated with UV before infection as compared to the survival of UV-irradiated phage in unirradiated cells (Witkin, 1976). The WR of phage has been associated with a high level of mutagenesis in the surviving phage (Weigle, 1953). This UV-induced mutagenic reactivation has been termed Weigle-mutagenesis (WM) and has often been used to quantify the error-prone nature of SOS induction in bacterial systems (Witkin, 1976). The expression or absence of phage WR and WM in normal and repair deficient strains of E. coli has helped elucidate the role of DNA repair in the molecular mechanism of UV-induced mutagenesis. Weigle (1953) was the first to describe the increased survival of UV-irradiated lambda phage after plating on host bacteria that were UV-irradiated. Among the reactivated phage, a large proportion were

mutants as judged by altered plaquing morphology. Using a lambda phage system, it was found that the UV dose relationships for clear plaque mutations and UV reactivation were extremely similar for each strain used (Defais et al, 1971). This suggested that there may be some step common to to the two processes. Mutant bacterial strains that were deficient in either (or both) rec A and lex A were also deficient in their ability to UV reactivate (WR) phage (Defais et al, 1971). In addition to UV, many chemicals and other physical agents (such as x- or gamma-rays) will induce the SOS response in bacterial cells as measured by enhanced reactivation (WR) (Witkin, 1976; Bresler et al, 1978; Hanawalt et al, 1979). The WR seen with gamma-irradiation of lambda phage was about half as efficient as the reactivation of UV-irradiated phage suggesting a different spectrum of mutational lesions induced by gamma-irradiation (Bresler et al, 1978).

Many of the UV-reactivation studies have used the double stranded DNA phage lambda as a probe which under certain conditions may integrate into the host chromosome becoming a lysogenic prophage. Since lambda is double stranded it can serve as a substrate for excision repair and therefore may present a more accurate representation of host repair function. The effect of the cellular level of rec A protein on the ability of E. coli K12 to either survive UV-irradiation, promote UV-reactivation (WR) or induce prophage was determined in bacterial mutants which had discrete increasing levels of recA protein (Quillardet et al, 1982). It was shown that a high basal level of Rec A due to constitutive expression (or plasmid amplification) of the rec A gene resulted in higher cellular UV

survival (Quillardet et al, 1982).

Cell and phage UV survival requires the expression of the umu C gene which also increases the survival of UV-damaged lambda in UV-irradiated host-cells (Ciesla, 1982; Quillardet et al, 1982; Shinoura et al, 1983b). A mutation in umu C prevented both spontaneous cellular mutagenesis and enhanced reactivation of lambda which was corrected through introduction of plasmid pKM101 (Ciesla, 1982). This plasmid expresses the muc A,B genes which are the functional equivalent of the chromosomal borne umc DC operon (Walker, 1984). The existence of two major mutational mechanisms have been suggested in E. coli: mispairing (misreplication) of DNA through erroneous base pairing directed by base analogs and mutagen modified bases; or misrepair mutagenesis involving DNA lesions which fail to pair (such as thymidine dimers) and block DNA replication (Shinoura et al, 1983). Agents that result in mispairing and misreplication have been called "direct" mutagens (Radman et al, 1979). Mutagens such as UV light have been termed "indirect" because of their dependence on the inducible. misrepair system (SOS) which both targeted and untargeted UV mutagenesis require (Radman et al, 1979; Witkin and Wermundson, 1979). The induction of a umu C mediated mutator process may be responsible for a lower base specificity as seen in mutational spectra produced with indirect mutagens as compared to direct mutagens (Coulondre and Miller, 1977). An excision deficient strain with a umu C⁻ background had an altered UV-induced mutational spectrum as compared to repair proficient umu C⁻ strains and it was concluded that the umu C gene exerts it's mutator activity via misrepair of DNA lesions provoking the

induction of all types of mutational events, though following UV-irradiation mainly transition events were seen (Shinoura et al, 1983). The umu C related studies has raised doubt about the existence of a unitary model of misrepair mutagenesis which predicts that all types of mutation, base substitution, frameshifts and deletions are the result of a common mutational mechanism (Lawrence and Christensen, 1979; 1982). For example, there appears to be two distinct modes of frameshift mutagenesis, one umu C dependent and similar to base substitution mutgenesis, the other umu C independent (Kato and Nakano, 1981; Shinoura et al, 1983b). The further use of phage probes in umu C⁻ as compared to umu C⁺ strains will be useful in judging the direct effects of rec A protein in WR and WM.

. The ability of the muc⁺ gene product of plasmid pKM101 to enhance cellular UV survival and mutagenesis lead to the finding that some SOS functions including WR and WM (of lambda phage) were enhanced by the expression of the muc⁺ gene (Walker, 1977; Walker, 1978; Swensen, 1981; Ciesla, 1982). Other SOS controlled functions such as prophage induction (Goze and Devoret, 1979) or synthesis of the RecA protein (Walker and Dobson, 1979) were not affected by expression of pKM101. It is clear that the muc⁺ gene product affects cellular processes in at least two ways either through enhancement of WR and WM or through protection against cell killing (Swensen, 1981). These two functions have not been separated as the result of Tn5 insertion at 21 sites in the muc gene so it has been suggested that the muc⁺ gene stimulates the expression of error-prone repair or that it actually codes for a SOS gene(s) (Shanabruch and Walker, 1980).

Mutations affecting the single-strand DNA binding protein (SSB) also impair the induction of mutagenic SOS repair (Whittier and Chase, 1983). The SSB⁻ strains have been shown to be deficient in WR and WM of lambda phage (Whittier and Chase, 1981) and it has been suggested that this protein is needed to participate directly in recombinational repair.

Another experimental approach to specifically study error-prone repair has been to use single stranded phage at low multiplicities thus avoiding recombinational and excision repair mechanisms (Froehlich, 1981; Schaaper and Glickman, 1982). WR of the SS DNA phage ϕX174 appears to be dependent on rec A expression and is independent of infecting multiplicities (Bleichrodt and Verheij, 1974; Das Gupta and Poddar, 1975). WR of the SS phage f1 does not seem to involve the recombinational activity of Rec A since no WR was seen in either mutant E. coli strain zab-53 (30% recombination proficient) or in lex A3 strain (60% recombination proficient) (Froehlich, 1981). WR of UV-irradiated f1 correlated with the presence of enhanced WM (Froehlich,1981). WR required high levels of rec A but increased levels such as those found constitutively in cells with spr-51 (allele of lex A) show no constitutive WR of f1 (Pacelli et al, 1979; Froehlich, 1981). This strongly implies that an activated form of rec A is necessary for the induction of WR of f1. Similarly, a direct participatory role of activated Rec A in mutagenesis has been demonstrated and is separate from the ability to derepress the SOS regulon (Witkin and Kogoma, 1984; Ennis et al, 1985).

Using the SS phage ϕR, WR and X-ray enhanced reactivation of

X-irradiated phage was lower than those for UV-irradiated phage (Ono and Shimazu, 1966). In a similar manner, UVER and XRER of lambda phage was used to examine the coupling of WR and WM in wild type and mutant E. coli strains (Bresler et al, 1978). Uncoupling of WR and WM was shown for UV-irradiated phage in a uvrE mutant such that WR was slightly depressed while WM was completely suppressed (as compared to wt) whereas with gamma-irradiated phage both WR and WM were deficient (Bresler et al, 1978). A lig-ts mutant of E. coli which is temperature sensitive for polynucleotide ligase was deficient in both WR and WM of UV and gamma-irradiated phage at the semi-permissive temperature of 37°C (Bresler, 1978). This result is very similar to the reduced UV-induced mutagenesis of bacteriophage T_4 mutants which possessed a ts ligase allele (Yarosh, 1978). It may be concluded that UV induces mutations through a pathway that includes polynucleotide ligase such as that proposed by Cupido (1983).

The UV-induced reversion frequency of six different bacteriophage M13 amber mutants for which the neighboring DNA sequences were known was determined in the presence or absence of Ag^+ ions which specifically sensitizes the DNA to dimer formation (Schaaper and Glickman, 1982). Parallel sensitization for both killing and mutagenesis of M13 was observed in the presence of Ag^+ suggesting that UV-induced mutagenesis is quantitatively correlated with dimer production (Schaaper and Glickman, 1982). Both WR and WM were clearly demonstrated using M13 (Schaaper and Glickman, 1982) and WR of single stranded phage M13 does not involve recombinational mechanisms since multiply infected cells W-reactivate to the same extent as singly

infected cells (LeClerc and Istock, 1984).

Error-prone repair mechanisms have been suggested as one of the major pathways of UV mutagenesis in bacteriophage T_4 (Bernstein, 1981). Phage T_4 has been useful in studying the molecular basis of UV mutagenesis for a number of reasons: extensive genetic mapping of T_4, good systems for scoring both forward and back mutations, and detailed knowledge of the enzymes involved in DNA replication (Drake, 1973; Bernstein, 1981). Temperature sensitive alleles of genes that function in DNA replication and repair have been implicated in the genetic determination of both spontaneous and UV-induced mutation rates (Drake, 1973). The alleles px, y and 1206 which are defective in genes uvsX, Y and uvsW respectively have been shown to inhibit UV mutagenesis (Green and Drake, 1974). Mutations in T_4 genes 30 (DNA ligase), td (thymidylate synthetase), v (pyrimidine dimer exonuclease) and hm (generalized repair) are also able to block error-prone repair of UV damaged T_4 (Drake, 1973; Yarosh, 1978; Bernstein, 1981). The error-prone bypass of pyrimidine dimers in DNA via induction of primer DNA has been found to be dependent upon gene 41 (RNA-priming protein), gene uvsZ (function unknown) and gene 30 (Cupido, 1983). Through the implication of ligase in error-prone repair of UV damage it is apparent that genes independent of replication may be involved in the bypass processes. It has therefore been proposed that phage T_4 codes for functions necessary for error-prone repair (Green and Drake, 1974).

It has been shown that the T_4 gene 43 (DNA polymerase) plays a central role in the fidelity of DNA replication with substantial effects of mutant polymerase alleles on mutation rates. Both mutator

and antimutator functions have been described for the T_4 DNA polymerase (Drake, 1969; 1974; Gillin and Nossal, 1976b; Clayton et al, 1979; Ripley and Shoemaker, 1983; Ripley et al, 1983). The biochemical properties of several of the mutator polymerases differ from each other suggesting that the underlying mutational processes may differ (Ripley and Shoemaker, 1983).

Genes 32 (helix destabilizing protein), 41 (RNA priming protein), 44 and 45 (both DNA pol accessory proteins) all strongly influence UV mutagenesis (Mufti, 1980). The observation that defective products of genes 41 and 45 cause wild type polymerase to be more accurate during repair of UV damage implies that gene 45 (as well as 41) may be involved in the error-prone bypass of T_4 thymidine dimers using primer RNA (Cupido, 1983). Taken together these results suggest that in T_4, UV-induced mutation occurs through an error-prone repair process which employs a multienzyme system involving at least 7 components including the DNA polymerase.

Multiplicity Reactivation of Damaged Bacteriophage

Multiplicity reactivation (MR) has been found to occur when two or more damaged phage enter a host cell and allow increased survival over that seen for singularly infected cells (Luria, 1947; Dulbecco, 1952). When UV treated T even phage were allowed to infect host cells at multiplicities between 0.01 and 0.95, the number of infective centers obtained was similar to the calculated number of doubly infected cells (Luria, 1947). UV-irradiation of T_4 gave MR values between 2.3 and 12.0 and the multicomplex (multiply infected cells)

inactivation curves had substantial shoulders (Bernstein, 1981). The interpretation of the shoulder is that there may be additional MR process which can be saturated when a certain number of lethal lesions has been reached (Nonn and Bernstein, 1977). Singly infected cells produce survival curves following the same kinetics as multicomplexes but without the shoulder (Bernstein, 1981).

MR of UV-inactivated phage T_4 requires the following phage genes: 32, 41, 44, 46, 47, 59 den V, uvs W, uvs S, y plus the host genes pol A and rec A (Bernstein, 1981). The above requirements for MR were determined by the decreased MR observed in mutants defective in these genes.

MR depends on host and phage recombination functions and produces a large effect when host excision repair is absent (Baker and Haynes, 1967; Huskey, 1969). Other phages including T_1, T_2, T_5, T_6, $\emptyset$X174 and VI; phage of S. typhi also undergo MR (Bernstein, 1981). The process of MR appears to be error-free in T_4 since it can overcome lethal lesions without inducing new mutations (Yarosh, 1978; Yarosh et al, 1980).

Host Cell Reactivation of Damaged Bacteriophage

Host cell reactivation (HCR) reflects the bacterial host cell's ability to carry out excision repair (Hanawalt et al, 1979). HCR has been found for the double stranded DNA phages T_1, T_3, T_5, T_7, but not for single stranded phages such as $\emptyset$X174 and S13 when assayed in HCR^+ host cells (Rorsh et al, 1964). Bacterial mutants that were HCR^- using lambda phage probes were found to be hypersensitive to the lethal

effects of UV-irradiation (Rorsch et al, 1964). The ability to eliminate thymidine dimers has been lost in HCR⁻ strains compared to HCR⁺ strains, therefore it is thought that excision repair defects may be responsible for this process (Boyce and Howard-Flanders, 1964; Boyle and Setlow, 1970). Phage T_1 damaged by UV light has been shown to undergo HCR that was dependent on the host genes uvrA, uvrB and uvrC (Howard-Flanders et al, 1966). The "short-patch" pathway of excision repair has been considered an "error-free" process since rec A⁻ and lex A⁻ mutants with competent excision are UV non mutable (Witkin, 1976).

Mismatch Repair

The mismatch repair process takes place on newly replicated bacterial DNA and is directed by the discrimination between newly replicated DNA which is non methylated as compared to the methylated parental strand (Lindahl, 1982; Bridges, 1983). This process has been described for phage lambda, φX174, f1 and the B. subtilis phage SPP1 that involves excision of a single strand segment of about 2,000 to 3,000 nucleotides (Radding, 1978). Heteroduplexes of these phage marked genetically on the two DNA strands have shown that incorrect base pairs can be eliminated from the heteroduplex prior to replication and that the products of E. coli genes mut H, mut L, mut S, and uvrE are required (Lindahl, 1982; Lu et al, 1983). Strains defective at these loci exhibit mutator phenotypes (Cox and Horner, 1983). The in vivo and in vitro activity of this repair system also depends on the dam directed methylation of specific sites in the genome (Lu et al, 1983). Deficiency or overproduction of the dam methylase results in a

mutator phenotype in a pathway that involves mut H, mut L, mut S (Glickman and Radman, 1980; McGraw and Marinus, 1980: Herman and Modich, 1981). The correction site (of considerable length) stretches between the mismatch site and the nearest dam methylation site and both must be present for mismatch repair to occur almost entirely on the unmethylated strand (Bridges, 1983; Lu et al, 1983). This repair system if operational, could significantly reduce error-prone repair processes through a post-replication surveillance process.

Photoreactivation Repair

The photoreactivation repair process utilizes photoreactivating enzyme (PRE) (photolyase) and visible light to monomerize dimers (Sutherland, 1978). Photoreactivation has been shown to reduce the yield of the UV-induced mutations suggesting that thymidine dimers may be mutagenic (Witkin, 1976). This finding has complicated the interpretation that the 6-4 photoproduct is a major premutagenic UV lesion (Haseltine, 1983) since PRE in vitro cannot reverse the 6-4 photo lesion. To accommodate these seemingly desparate results Haseltine (1983) suggests that thymidine dimers are major inducers of SOS repair which is absolutely required for the fixation of mutations at 6-4 photo lesions. Therefore photoreactivation may reflect reversal of the "signal" producing (but not premutational) lesions required for SOS induction.

II. UV Repair in Eukaryotic Cells

Excision Repair of UV-damaged DNA

The major pathways of repair found in E. coli are similar to those described for mammalian cells and no novel DNA repair pathways have been found unique to the more complex structures of eucaryotes (Lindahl, 1982). Both nucleotide and base excision repair mechanisms have been found in mammalian cells (Grossman, 1978; 1981; Friedberg et al, 1981). Excision repair requires the action of a specific endonuclease to recognize lesions and incise DNA adjacent to the damaged sites. In the yeast S. cerevisiae at least five genes are required for the incision of UV-irradiated DNA in vivo (Reynolds et al, 1981; Wilcox and Prakash, 1981). Mutants defective in these genes are highly sensitive to UV-radiation and totally defective in DNA incision, and pyrimidine dimer excision repair (Naumovski and Friedberg, 1983).

Similarly, deficiences in excision repair have been characterized in human cells from the genetic syndrome xeroderma pigmentosum (XP) (Friedberg et al, 1979). The complexity of XP is highlighted by the identification of nine complementation groups in excision repair-deficient XP cells designated A to H (de Weerd-Kastelein et al, 1973; Paterson et al, 1974; Kraemer et al, 1975; Takebe, 1978; Bootsma, 1978; Arlett et al, 1980; Moshell et al, 1983). The process of excision repair appears to require at least three different biochemical processes, nucleolytic mediated removal of damaged DNA, resynthesis of the damaged strand using complementary DNA as template, and ligation of the newly synthesized patch of DNA

(Kaufmann et al, 1983). Thus, excision repair of UV damage is a complex sequence of steps involving the recognition of damage and the action of endonucleases, exonucleases, polymerases and ligases together with other factors which are associated with changes in chromatin (Cleaver, 1983). DNA excision repair in human cells has been classified into two forms, depending on the nature of the initial insult to the DNA (Regan and Setlow, 1974). Damage by UV (or other agents causing bulky lesions) results in the excision of 15-25 bases (Smith and Okumoto, 1984). The latter occurs during an 18 to 20 hour repair period whereas ionizing radiation damage is repaired by the insertion of 3 to 4 nucleotides during a brief period (2 hour) after the insult (Regan and Setlow, 1974). It should be noted that long patch repair in E. coli. involves excision of much longer patches (> 100 nucleotides) and is SOS dependent (Hanawalt et al, 1979). These processes have been respectively termed "long" patch and "short" patch repair. Aphidicolin treatment indicated that pol alpha was the dominant excision polymerase which fills the single strand gap left after a complex of polypeptides recognizes, cleaves and excises the damaged oligonucleotide in a "cut and patch" model (Cleaver, 1983).

In UV-irradiated cells, the patches of newly synthesized DNA within chromatin display increased sensitivity to digestion by micrococcal nuclease (Cleaver, 1977; Smerdon et al, 1980). The sensitivity of the patches decreases with time suggesting that nucleosomes are disassembled during or before the DNA resynthesis step and reassembled after the gap filling is completed (Smerdon and Lieberman, 1980; Bodell and Cleaver, 1981).

Eukaryotic Post Replication Repair

Eukaryotic (mammalian) post replication repair (PRR) mechanisms have been proposed to account for "repair" or tolerance of lesions produced in actively replicating regions of the genome (Hanawalt et al, 1979; Hall and Mount, 1981). Alkaline sucrose gradient analysis of UV-irradiated cellular DNA have revealed that initially small nascent DNA molecules are converted to parental size after several hours (Lehmann, 1972; 1974). Evidence has accumulated to suggest that different processes may be responsible for PRR, either a) replication may be blocked in the nascent strand complementary to the parental strand containing the damage, eventually the "pause" is overcome and elongation continues; b) damage initially blocks replication but reinitiation occurs beyond the dimer leaving a gap, which is subsequently filled by de novo synthesis (Hall and Mount, 1981); c) inhibition of initiation occurs in replicons by damage anywhere in the replicon (or clusters of replicons) or by d) cessation of DNA synthesis in replicons due to the presence of damage anywhere in the replicon (Lehmann and Karran, 1981).

Studies on the structure of the newly synthesized DNA in UV-irradiated mammalian cells uniformly suggest that there is temporary blockage of DNA replication forks at dimer sites however there is much controversy (depending on cell type and experimental design) on how this is overcome (Hall and Mount, 1981). Extensive recombinational exchanges do not seem to be associated with mammalian PRR (as they are in bacteria) however, branch migration followed by synthesis of the

damaged region using the complementary daughter strand is a likely
mechanism that would allow lesions to be tolerated (Hanawalt et al,
1979; Hall and Mount, 1981). The size of the DNA strands made after
UV-irradiation is roughly comparable to the distance between dimers
suggesting that SS gaps form in daughter DNA strands (Lehmann and
Karran, 1981). These gaps are sensitive to SS specific endonuclease
and disappear about 2h after UV-irradiation of WI 38 normal fibroblasts
(Meneghini, 1976). If de novo DNA synthesis occurs opposite a
non-instructional dimer, then gap filling may be error-prone (Paterson
and Smith, 1979). XP variant cells (see below) which have abnormal PRR
(Cleaver et al, 1981) also are UV hypermutable (Maher et al, 1976) and
strongly implies that deficient PRR allows the expression of an
error-prone repair process.

Human Autosomal Recessive DNA Repair Deficient Syndromes

Xeroderma Pigmentosum

Xeroderma pigmentosum (XP) is a rare autosomal recessive
disorder characterized by hypersensitivity of exposed skin to sunlight
with subsequent hyperpigmentation, degeneration and increased risk of
neoplasia in exposed areas (Kraemer, 1977; Friedberg et al, 1979;
Bridges, 1981). There are two clinical forms of the disease expressed
either as the "classical" form with only skin complications, and the
"neurological" form which also exhibits degeneration associated with
the central nervous system (Robbins et al, 1974; Kraemer, 1977;
Paterson, 1979; Bridges, 1981). Skin fibroblasts taken from these
patients have been classified into nine distinct genetic forms of XP;

(groups A-H) which are defective in the excision repair of pyrimidine dimers, and the variant which is defective in daughter strand repair (Lehmann et al, 1975; Friedberg et al, 1979; Paterson et al, 1984).

The relationship between the in vivo hypersensitivity of XP patients to the UV component of sunlight and the DNA repair deficiency of UV-irradiated XP fibroblasts in vitro has been well established (Cleaver, 1968; Cleaver, 1970; Kraemer, 1977). At the cellular level, an increased sensitivity to far UV (254 nm) is indicated by the reduced colony forming ability (as compared to normals) which varies between the different complementation groups (Kraemer, 1977; Bootsma, 1978). Non-dimer damage may also be responsible for cell killing after near UV-irradiation and XP group D cells appear to be the complementation group most compromised in the ability to repair this type of damage (Smith and Paterson, 1982). Treatment with the UV-mimetic compound MMS produces a lower colony-forming ability in XP cells but a normal UDS suggesting that there is no correlation between cellular survival and UDS (Cleaver, 1971; Cleaver, 1973; Stich et al, 1973; Cleaver, 1977; Witte and Thielmann, 1979; Thielmann and Witte, 1980). XP cells display fewer alkaline labile sites (compared to normal) in their DNA following alkylation with MMS, EMS or isopropyl methanesulfonate suggesting that there may be a deficiency in a glycosylase or apurinic endonuclease (Snyder and Regan, 1982). There is also a deficiency in the ability of XP cell-free extracts to incise methylated DNA (Witte and Thielmann, 1979; Thielmann and Witte, 1980). These enzyme defects may not be related to repair processes since it has been found that XP cells can repair MMS treated SV40 DNA to a greater extent than normal

cells (Kudrna et al, 1979) and the reported efficiency of XP cells to remove O^6-alkylguanines from their DNA may be dependent on the transformed state rather than on the XP mutation itself (Goth-Goldstein, 1977; Bodell et al, 1979; Day, 1980; Teo et al, 1983).

XP cell survival following treatment with ionizing radiation is similar to normal cells (Sasaki et al, 1977; Arlett, 1980), with the exception of XP3BR (group G) which has increased sensitivity to gamma irradiation (Arlett et al, 1980). Similarly, XP cells are not hypersensitive to the killing effects of radio-mimetic compounds (Arlett and Lehmann, 1978).

The XP variant presents a clinical picture similar to the classical XP; however, at the cellular level in vitro, XP variants appear to perform normal amounts of excision repair as measured by UDS but have reduced colony forming ability following UV-irradiation (Friedberg, 1979; Cleaver et al, 1981; Paterson et al, 1984). The defect common to XP variants is the inability to replicate DNA without interruption at damaged sites such that replication forks are blocked more frequently and at a lower dose in variant cells as compared to normals (Lehmann et al, 1975; Cleaver et al, 1979; 1980).

The mutagenic effects of UV-irradiation in a normal and two excision deficient XP strains (XP2BE, XP12BE) (Maher and McCormick, 1976) indicated the two XP strains demonstrated increased UV-induced mutation per unit dose over normals but when corrected for survival there was no difference between the XP and normal strains (Maher and McCormick, 1976). Mutagenicity is therefore directly related to the cellular cytotoxicity of UV, both of which can be eliminated by

excision repair and strongly suggest that excision repair is an error-free process (Maher and McCormick, 1976; Maher et al, 1979). XP variant cells have both a higher frequency of UV-induced mutations per unit dose and per survivor when compared to normals and suggests that the defect in XP variants results in expression of an error-prone repair process (Maher et al, 1976). The existence of an error-prone repair in XP cells correlated with an increased cancer incidence in these patients is a persuasive arguement for the somatic theory of carcinogenesis.

Cockayne's Syndrome

Cockayne's syndrome (CS) is an autosomal recessive disorder characterized clinically by growth retardation, skeletal and retinal abnormalities, neurological defects, mental retardation and sun sensitivity and as with XP these fibroblasts _in vitro_ are hypersensitive to UV (Arlett and Lehmann, 1978; Bridges, 1981; Lehmann, 1982). This syndrome does not have XP-like freckling or an increased risk of cancer among patients (Guzzetta, 1972).

In normal cells both RNA and DNA synthesis is depressed by UV-irradiation but recovers within hours whereas in CS (and XP), depression of RNA and DNA synthesis is seen with no recovery (Lehmann et al, 1979; Mayne and Lehmann, 1982).

A large increase in the accumulation of long lived spontaneous DNA breaks was found in CS cells when compared to normal (Squires and Johnson, 1983). CS fibroblasts have a reduced ability to reactivate UV-irradiated adenovirus 2 or 5 (see HCR of mammalian viruses) (Day et

al, 1981; Rainbow and Howes, 1982) suggesting a repair defect.

Cell fusion studies have shown that there are at least three complementation groups among isolated CS strains (Lehmann, 1982) suggesting genetic heterogeneity, and a repair defect is suggested by the wide range of sensitivity to UV seen among different CS strains (Andrews et al, 1978).

CS fibroblasts have been found to be hypermutable when the cellular UV induced mutation freqency (to 6 - thioguanine resistance) was compared to normals (Arlett, 1980). One CS strain was more mutable than normal strains at a given UV dose while with another strain (11961) mutability was slightly higher than normals (Arlett, 1980). The somatic mutation theory of carcinogenesis suggests that CS patients which are not cancer-prone, should not be hypermutable however, it has been suggested that the lack of an increased risk of neoplasia in CS may be the result of premature death of afflicated individuals (Bridges, 1981).

Ataxia Telangiectasia

Ataxia telangiectasia (Louis-Barr Syndrome, abbreviated as AT) is a rare human autosomal recessive disorder characterized by an abnormally high sensitivity to ionizing radiation (Paterson and Smith, 1979; Bridges, 1981; Huang and Sheridan, 1981). The clinical abnormalities associated with AT are complex and pleiotropic with the following cardinal characteristics. A progressive cerebellar ataxia can be seen as an early symptom followed by a later onset of oculocutaneous telangiectasia (Huang and Sheridan, 1981). Aberrant

embryonic differentiation has been suggested to explain both impaired organogenesis and immunological deficiencies (Peterson et al, 1964; McFarlin et al, 1972) with AT patients having a small or absent thymus, and increased levels of serum a-fetoproteins suggesting that the liver is not fully developed (McFarlin et al, 1972; Waldmann and McIntire, 1972). Both cell-mediated and humoral immune responses are abnormal in AT (McFarlin et al, 1972; Waldmann et al, 1983).

Another hallmark of AT is an elevated risk of lymphoreticular malignancy (Arlett and Lehmann, 1978; Paterson and Smith, 1979). About one in ten AT patients develops cancer which is a frequency approximately 120 fold greater than that in an age matched control population (Paterson et al, 1984). AT heterozygotes also have about a 5x increased likelihood of developing fatal malignancy before age 45 as compared to a control population (Swift et al, 1976).

Radiosensitivity to ionizing radiation observed clinically has been clearly demonstrated in vitro using cultured AT fibroblasts and peripheral blood lymphocytes. AT fibroblasts have been found to be uniformly hypersensitive to ionizing radiation using the loss of colony forming ability as an assay (Taylor et al, 1975; Arlett and Harcourt, 1980; Ikenage et al, 1983; Nagasawa and Little, 1983). A close relationship has been found between the production of chromosomal aberrations and cell killing by moderate doses of X-irradiation in cultured mammalian cells (Dewey et al, 1971; Carrano, 1973a, b; Carrano and Heddle, 1973; Bedford et al, 1978; Nagasawa and Little, 1981). This relationship has been confirmed in AT fibroblasts and lymphocytes which exhibit a higher frequency of X-ray induced chromosomal

aberrations when compared to normals (Highurashi and Conen, 1973; Rary et al, 1974; Taylor et al, 1976; Taylor, 1978; Natarajan and Meyers, 1979; Zampetti-Bossler and Scott, 1981; Nagasawa and Little, 1983).

An elevated level of spontaneous chromosomal abberations have also been reported in AT fibroblasts and lymphocytes (Gropp and Flatz, 1967; Hecht et al, 1973; Oxford et al, 1975; Taylor et al, 1976; Cohen et al, 1978; Cohen et al, 1979). In cytogenetic studies, nonrandom rearrangements have been observed in AT lymphocytes and fibroblasts. Various anomalies involving chromosome 14, 7 or both have been described (Hecht and McCaw, 1973; Oxford et al, 1975; McCaw et et, 1975; Aurias et al, 1980).

There have been many attemps to find a biochemical DNA-repair defect which is common to all AT cells after it was suggested that AT is a defective X-ray repair analog of xeroderma pigmentosum (Paterson and Smith, 1979; Bridges, 1981; Huang and Sheridan, 1981). At the level of DNA repair, AT cells have been shown to be capable of normal rejoining of x-ray induced single and double standed breaks (Taylor et al, 1975; Lehmann and Stevens, 1977; Forance and Little, 1980) and have been divided into classes depending on their ability to express x-ray induced excision repair (Paterson and Smith, 1979; Vincent et al, 1980). One class designated exr⁻, demonstrate reduced levels of x-ray - induced DNA repair synthesis or reduced UDS and the slow removal of base sugar damage while exr⁺ are competent for x-ray - induced excision repair (Paterson and Smith, 1979).

Recently the observation has been made that DNA synthesis as measured by ^{3}H-thymidine incorporation is not inhibited in AT cells

after ionizing radiation (Houldsworth and Lavin, 1980; Edwards and Taylor, 1980; Painter and Young, 1980; Painter et al, 1982; Painter and Young, 1982; Painter, 1983). This is not the case in normal human cells in which moderate doses of ionizing radiation severly inhibit the rate of DNA synthesis while the radioresistant DNA synthesis of AT seems to be a hallmark of this syndrome (Painter, 1981). It has been suggested that altered conformation of chromatin is responsible for the radioresistant DNA synthesis which is seen in both AT cells and in caffeine treated normal cells (Walters, et al, 1974; Tolmach et al, 1977; Painter, 1980).

AT cells have been found to be mainly normal with respect to cell killing by UV light (Taylor et al, 1975; Cox et al, 1978; Arlett, 1980) but it has been reported that skin fibroblasts from AT, show an increased susceptibility to light induced chromatid breakage (Parshad et al, 1981). Repair enzymes involved in the removal of UV-induced lesions and the "UV-like" AAF adduct appear to be normal in AT cells (Amacher and Lieberman, 1977; Arlett and Lehmann, 1978).

An unusual feature of AT fibroblasts is an apparent reduction in cellular mutation frequency following x-irradiation (Arlett and Lehmann, 1978). The hypomutability of AT fibroblasts was demonstrated when gamma-induced mutations to 6-thioguanine resistance were measured in normal and repair deficient cell strains (Arlett, 1980). This study showed that the mutation frequency per survivor was decreased for AT cells as compared to normal or XP cell strains. In a similar experiment both CS and XP cell strains were hypermutable compared to normals following UV-irradiation while AT cell strains were slightly

elevated at high UV doses. Arlett, (1980) concluded that AT may be defective in an "error-prone" repair process expressed in normal and XP cell strains.

Host Cell Reactivation (HCR) of Damaged Mammalian Viruses

The reactivation of damaged virus in uninduced cells has been termed HCR and has been quantified in repair proficient and deficient cells using a number of different viruses and different viral functions (Rainbow, 1981). HCR has been characterized by the following viral functions: plaque formation of HSV (Lytle, 1971; Takebe et al, 1974); SV40 (Abrahams and Van der Eb, 1976) and adenovirus (Rainbow and Mak, 1973; Day, 1974; Day, 1975; Hoar and Davis, 1979; Day et al, 1981); intranuclear inclusion body formation of Ad (Rainbow and Mak, 1972; Stich et al, 1974; Stich, 1975); T antigen formation and transformation frequency of SV40 (Aaronson and Lytle, 1970); Ad viral structural antigen (Vag) formation (Rainbow, 1978); repair of Ad DNA lesions and HSV progeny formation after single cycle growth (Rabson et al, 1969; Coppey et al, 1978).

Abnormally reduced HCR has been shown for many repair deficient human strains using the techniques described above (for review see Rainbow, 1981). XP cells are severely inhibited in HCR of UV-irradiated adenovirus (Day, 1974; Rainbow and Howes, 1979; Rainbow, 1980; Rainbow, 1981), as well as UV'D HSV (Rabson et al, 1969 and Lytle et at, 1972; Ryan, 1983) and SV40 (Aaronson and Lytle, 1970). Similar values for HCR in XP strains have been found using either plaque formation or Vag of adenovirus such that the reduced HCR values

correlate well with the reduced levels of UDS found in the different XP complementation groups with the exception of group D which shows far more UDS than HCR ability (Rainbow, 1981). The XP variant strain has been found to be slightly reduced in HCR (Day, 1974; Rainbow and Howes, 1979; Rainbow, 1981). This suggest that post-replication repair of UV damage is partially responsible for HCR. XP heterozygotes have also been found to be reduced in the HCR of UV-irradiated Ad when compared to normal fibroblasts (Rainbow, 1980). Since XP heterozygotes have an elevated incidence of skin cancer (Swift and Chase, 1979) there may be a direct link between the DNA repair defect and cancer proneness (Rainbow, 1980).

Cockayne's syndrome (CS) fibroblasts have been reported to display reduced HCR of UV-irradiated adenovirus (Day et al, 1981; Rainbow and Howes, 1982). A reduced HCR of Vag formation has also been found for gamma-irradiated Ad2 in CS fibroblasts (Rainbow and Howes, 1982) and in XP strains (Rainbow and Howes, 1979) suggesting that a proportion of the gamma-ray damage is "UV-like". Using X-irradiated HSV as a probe, Lytle et al, 1972 found that HCR was only slightly reduced in XP cells as compared to normals. In the previous two examples, virus was irradiated in the frozen state, whereas if ^{60}Co-irradiation of HSV occurs in a liquid state, virus survival in XP cells is reduced (as compared to virus irradiated in a frozen state) but not significantly decreased from normal HCR levels (Zamansky and Little, 1982). These results suggest that direct ionizing radiation effects (frozen) may produce more "UV-like" damage than when indirect effects (liquid irradiation) predominate. No abnormal HCR (using HSV

as a probe) was found in two other XP strains, ataxia telangiectasia, hereditary retinoblastoma, Gardner's syndrome, Progeria or Fanconi's anemia (Zamansky and Little, 1982). The smaller relative reductions in HCR of UV-irradiated HSV in XP as compared to adenovirus suggests that the more complex genome of HSV may code for functions that supplement UV-repair (Lytle et al, 1972; Day, 1974). In CS, the HCR of UV-irradiated adenovirus is substantially reduced; however, the HCR is near-normal in CS using UV-irradiated HSV, possibly suggesting a HSV induced process not found in adenovirus infected cells which alleviates the repair defect in CS (Day et al, 1981; Rainbow and Howes, 1982; Ryan, 1983).

Mammalian viruses have been used to probe the DNA repair defect associated with AT. The host cell reactivation of UV-irradiated adenovirus was found to be 50-70% reduced in AT fibroblasts as compared to normal strains (Rainbow, 1978). Using EBV-transformed lymphoblastoid cell lines from AT patients and gamma-irradiated HSV as a probe, no deficiency in X-ray incision repair was found even though these cell lines were sensitive to x-irradiation as assayed by reduced colony formation (Henderson and Long, 1981). The normal HCR of Ataxia lymphoblastoid cell lines was recently confirmed using HSV x-irradiated under anoxic conditions (Henderson and Basilico, 1983). These authors suggest that the normal x-ray excision repair of x-irradiated HSV supported the concept that the defect in AT resides in mechanisms other than DNA repair. If the AT defect is the result of altered chromatin conformation resulting in reduced accessibility of repair enzymes to DNA as suggested by Smith and Paterson (1983) then it is possible that

HSV DNA replicates independently from host chromatin thus allowing normal access of functional repair enzymes.

Multiplicity Reactivation of Damaged Mammalian Viruses

Multiplicity reactivation (MR) of phage has been hypothesized to consist of recombinational exchanges between 2 or more lethally damaged genomes thereby allowing increased survival of the damaged phage (Huskey, 1969). A similar phenomenon has been reported for UV-irradiated mammalian virus (Selsky et al, 1979; Hall et al, 1980). It is not clear if the higher survival found at high moi is the result of increased recombinational exchanges; however, it has been reported that UV-irradiation stimulates the recombination frequency of SV40 or HSV (Dubbs et al, 1974; Das Gupta and Summers, 1980; Hall et al, 1980).

Multiplicity reactivation of HSV treated with the alkylating agents MMS and MNNG (but not HN_2) occurred in Vero cells and in the normal human fibroblast line FS2 (Das, 1982). The second resistant component observed in HSV UV survival curves has been hypothesized to be the consequence of MR; however, MR or an inhomogeneous virus population was determined not to be responsible (Lytle, 1971).

Enhanced Viral Reactivation, Enhanced Viral Mutagenesis and Eukaryotic Error - Prone Repair

The realization that, following UV damage in E. coli., increases in both cellular and phage survival (with accompanying mutagenesis) requires SOS processing has initiated an effort to identify a similar process in mammalian cells. There is a large body of literature

describing the enhanced reactivation (ER) of damaged mammalian viruses in cells that have been pretreated with DNA damaging agents (for review see Lytle, 1978; Rainbow., 1981). Enhanced reactivation of UV-irradiated or X-ray irradiated virus in monkey kidney cells is limited to nuclear replicating viruses since both DNA or.RNA containing viruses which are cytoplasmic replicating cannot express ER phenomenon (Bockstahler and Lytle, 1977).

ER of UV-irradiated mammalian viruses has been demonstrated for SV40 (Bockstahler and Lytle, 1977; Sarasin and Hanawalt, 1978; Cornelis et al, 1980; 1981; Gentil et al; 1982; adenovirus (Jeeves and Rainbow, 1979; 1983a; 1983b; 1983c), herpes simplex virus (HSV) (Bockstahler and Lytle, 1970; 1971; 1977; Das Gupta and Summers, 1978; Lytle and Goddard, 1979; Coppey and Menezes, 1981; Nocentini et al, 1981; Takimoto et al, 1982; Glazier et al, 1982; Schnipper et al, 1983; Ryan, 1983), Kilham rat virus (Lytle 1978a), minute virus of mice (Vose et al, 1981; Rommelaere et al, 1981; Yu et al, 1982; Rommelaere and Ward, 1982), and parvovirus H1 (Su et al, 1981) using such agents as UV, x-rays, aflatoxin B1, AAAF, MMS, hydroxyurea, phosphonoacetic acid, ara A or ara C as an inducing agent. ER is the mammalian equivalent of Weigle reactivation and as such, may represent a mammalian SOS response to cellular DNA damage. In the presence of cycloheximide which blocks de novo protein synthesis, ER is decreased in UV or carcinogen induced cells (Das Gupta and Summers, 1978; Lytle, 1978a; Lytle and Goddard, 1979, Sarasin and Hanawalt, 1978; Su et al, 1981). ER values also increase with increasing time delays between cellular DNA damage and viral infection suggesting that ER is an inducible process possibly

requiring protein synthesis (Bockstahler et al, 1976; Lytle et al,
1976; Lytle, 1978a; Cornelis et al, 1981; Rommelaere et al, 1981; Su et
al, 1981; Glazier et al, 1982; Jeeves and Rainbow, 1983a; 1983b;
1983c).

ER has been found in cells from a variety of origins including
human, monkey, rodent and marsupial (Lytle, 1978a; Rainbow, 1981) and
the level of ER depends not only on the cell type but also on the viral
system and conditions of cell culture and irradiation (Lytle, 1976;
Bockstahler and Lytle, 1977; Lytle, 1978). Comparisons between viral
systems and cell species is made difficult by the differences both in
viral replication complexity and host range specificity of replication;
for instance, HSV can infect a broad range of cells exhibiting
different levels of ER depending on the cellular host (Lytle, 1978).

Day and Ziolkowski (1978, 1981) have measured the reversion
among progeny resulting from infections of intact or UV-irradiated
adenovirus (Ad5ts2) in UV-irradiated or unirradiated A498 cells (human
kidney tumor) or CRL1187 (normal human fibroblasts). Enhanced
mutagenesis was not seen among the progeny of UV-irradiated Ad5ts2 if
the host cells received UV (5 J/m^2) 20 hour before infection (Day and
Ziolkowski, 1981). The number of revertants increased in a UV-dose
dependent manner (Day and Ziolkowski, 1981). It was suggested that the
lack of EM may be due to the multiplicity of infection used (moi of
about 1) during the experiments which allowed an error-free
multiplicity reactivation to occur) (Day and Ziolkowski, 1981).
Similarly, UVEM of UV-irradiated Ad2 was not seen in HeLa cells
UV-irradiated 18h before infection (Piperakis and McLennan, 1984).

Much interest in inducible mammalian error-prone repair has been generated from the observation that in CV-1 monkey kidney cells UV-irradiated or treated with chemical carcinogens, there was a large increase in reversion of UV-irradiated SV40 tsA or ts B mutants as compared to that in uninduced cells (Sarasin and Benoit, 1980; Sarasin et al, 1981; Sarasin et al, 1982). These authors concluded that enhanced virus survival was associated with a relaxed fidelity of repair or replication leading to a higher mutation frequency in the viral progeny. This occurs for UV-irradiated virus when grown in CV-1 cells that have been inhibited in DNA replication (Sarasin et al, 1982) presumably by an error-prone by-pass of UV-lesions which may be the expression of an "SOS"-like response (Sarasin and Benoit, 1980).

A similar experiment using UV-irradiated and intact SV40tsBC245 grown for one cycle at the permissive temperature in UV-induced or uninduced BSC-1 monkey cells revealed a UVER that was maximally expressed at 3 days post irradiation which was not accompanied by EM (Cornelis et al, 1980). Another study from the same group again failed to find a UVEM response with the same virus and cells but did find a strong untargeted mutator effect that was inducible with maximal expression at 72 hours post-irradiation (Cornelis et al, 1981).

Taylor et al (1982) examined CV-1 cells for existence of a UVEM of UV-irradiated SV40 using the same experimental procedure as Sarasin and Benoit (1980). In two separate laboratories, UVEM of UV-irradiated SV40tsB201 was not found in CV-1 monkey kidney cells even though UVER was found in experiments carried out in parallel (Taylor et al, 1982). The previous authors suggested that UVER and UVEM are separate

processes, arising from different mechanisms but were unable to account for the discrepancy between their data and those of Sarasin and Benoit (1980).

HSV has also been used as a probe of cellular error-prone repair and it should be realized that as a probe of cellular DNA synthesis, it is the most complex and the least dependent on host function to replicate. This virus is known to code for or induce several enzymes involved in DNA metabolism including DNA polymerase (Purifoy et al, 1977), thymidine kinase (Wigler et al, 1977), ribonucleotide reductase (Huszar and Bacchetti, 1981) and alkaline DNase (Francke and Garrett, 1982). UVEM studies with this virus must be interpreted with the realization that viral coded functions may be actively participating in the repair/replication process.

Das Gupta and Summers (1978), measured the UV-induced forward mutation of wild type (TK^+) to TK deficiency (TK^-) among the progeny of HSV-1 grown in Vero cells that had been UV-irradiated or left unirradiated. Inducible untargeted and targeted responses both of which paralleled enhanced reactivation were found to peak when viral infection was delayed 16 hours after cellular irradiation (Das Gupta and Summers, 1978), and significant UVEM was not found. Cycloheximide treatment of cells in the interval between irradiation and infection was found to decrease UVER of HSV-1 suggesting the need for de novo protein synthesis to express this phenomenon (Das Gupta and Summers, 1978). Error-prone repair of UV-irradiated or unirradiated HSV-1 in UV-irradiated or unirradiated CV-1 monkey kidney cells was studied by measuring the forward mutation of HSV wt virus (TK^+) to

Iododeoxycytidine (ICdR) resistance (TK⁻) (Lytle et al, 1980). An untargeted increase in mutagenesis was seen for undamaged virus in cells which were UV-irradiated 24 hours before infection (Lytle et al, 1980). Targeted mutagenesis increased in UV-irradiated as compared to nonirradiated cells. The magnitude of this targeted increase (and UVEM) was dependent on the moi such that increased UVEM was concomitant with increased moi. A maximal UVEM of 0.95 did not indicate the existence of an error-prone repair (as calculated from Lytle et al, 1980). The lack of UVEM in the presence of UVER is in agreement with the findings of Bockstahler et al, 1981 using a HSV ts mutant and suggest UVER and UVEM may arise by separate processes (Bockstahler, 1981; Bockstahler et al, 1981). Similarly, Takimoto (1983), measured the frequency of the forward mutation to ICdR resistance for unirradiated, UV-irradiated or gamma-irradiated HSV grown in UV-irradiated or unirradiated CV-1 cells. No UVEM was seen, even under conditions which maximized the expression of UVER for this virus (Takimoto, 1983).

Another study using HSV-1 as a probe of mutagenic repair in an SV40 transformed human cell line, NB-E, revealed a positive UVEM at high dose to virus (UVEM values of approximately 1.8, and 2.2 as calculated from Lytle and Knott, 1982). Plotting log induced mutation frequency versus the log of UV fluence revealed that the slope for unirradiated virus was 1.1 and for UV-irradiated virus was 1.3, suggesting that 1 "hit" was required to produce an ICdR resistant mutant in HSV (Lytle and Knott, 1982). The targeted and untargeted increase of mutations for HSV in the NB-E cell line was shown to

parallel the UVER of virus in a dose dependent manner (Lytle and Knott, 1982). The previous data is suggestive that UVER in NB-E is accompanied by increased mutagenesis of virus.

The forward mutagenesis of UV-irradiated HSV-1 was measured at the viral TK locus in uninduced normal, XP group A or XP variant fibroblasts (Lytle et al, 1982). The mutation frequency of progeny HSV increased in a dose dependent linear fashion for the XPA and normal strain however, the XP variant produced HSV progeny with mutation frequencies that increased as a square of the UV dose (Lytle et al, 1982). The XPA strain produced a mutation frequency greater than normal cells when compared on a per unit dose basis but were similar when compared per lethal hit (Lytle et al, 1982). The previous study suggests that excision repair is an error free mechanism.

HSV-1 forward mutagenesis ($TK^+ \longrightarrow TK^-$) of intact virus was used to examine UV-induced viral mutagenesis and reactivation in normal and XP fibroblast strains (Abrahams et al, 1984). The time course of UVER paralleled that for enhanced mutagenesis (EM) of intact virus in normal, XP complimentation groups A,C and D but not in XP variant fibroblasts. Maximal EM and UVER were transiently expressed when infections were delayed 1 or 2 days after cellular irradiation (Abrahams et al, 1984). Separate time courses of EM and UVER in XP variants suggests that these are independent phenomena in these cells (Abrahams et al, 1984).

Mutation of HSV to ICdr resistance was used to characterize normal skin fibroblasts, tumor cells and skin fibroblasts from donors that had also submitted osteoscarcoma tumor cells (Bockstahler et al,

1982). Replication of undamaged virus was more mutagenic in lung carcinoma, malignant melanoma and 2 osteosarcoma cell lines when compared to normal skin fibroblasts (Bockstahler et al, 1982). Skin fibroblasts from the 2 osteosarcoma patients and a glioblastoma tumor cell line had HSV mutation frequencies similar to normal fibroblasts (Bockstahler et al, 1982). This suggests that some but not all tumor cell lines have a somatically activated mutator function possibly related to the oncogenic state.

The SS autonomous parvovirus H-1 has also been used to study both the targeted (Cornelis et al, 1982) and untargeted (Cornelis et al, 1981) mutagenesis of viral growth in cells that had been UV-irradiated. NB-E cells that had been pre-irradiated with UV or preinfected with UV-irradiated SV40, showed a transient increase in untargeted mutagenesis of H-1 ts6 that peaked when the time interval between cell treatment and H-1 infection was about 15 hours (Cornelis et al, 1981). It is of particular interest that exogenously introduced damaged DNA in the form of either UV-irradiated SV40, ϕX174, H-1 or DS DNA from calf thymus could induce this untargeted mutator activity suggesting that damage need not be cellular to induce this activity (Cornelis et al, 1981; Cornelis et al, 1982; Dinsart et al, 1984). The enhanced untargeted response was twice that seen in unirradiated cells and peaked at 3 J/m^2 to the cells (Cornelis et al, 1981). Targeted and untargeted reversion of H-1 ts6 was determined in UV-irradiated or nonirradiated NB-E cells over a range of input multiplicities such that at high moi (about 0.5 pfu/cell or higher) parallel decreases in mutation frequencies were seen for both targeted and untargeted

responses (Cornelis et al, 1982). This was interpreted as being the
result of fewer allowable rounds of replication of mutant virus in
multiply infected cells (Cornelis et al, 1982). The frequency of
mutations resulting from cellular UV-irradiation (14 hour delayed viral
infection) was the same or only slightly higher for UV-irradiated virus
over undamaged virus (Cornelis et al, 1982). This suggests that over
the dose range used, there was no UVEM but a strong (2x) untargeted
mutator response in the transformed human cell line (Cornelis et al,
1982). Treatment of transformed rat or human (NB-E) cells with
2-nitronaphthofuran derivatives resulted in the concomitant expression
of both a cellular untargeted mutator activity (using H-1ts 6 as a
probe) and enhanced survival (Su et al, 1981). The activation of both
functions was transient, peaking when infection of virus was delayed 14
hours after cell treatment (Su et al, 1981). This suggests that ER and
EM (untargeted) in nitronaphthofuran induced cells may be both
dependent on de novo protein synthesis but represent separate processes
since ER peaked at a 2-nitronaphthofuran concentration of 0.1 mg/ml
while EM of UV-irradiated virus peaked at 2 ug/ml (Su et al, 1981).

A summary of the parameters of error-prone repair in mammalian
cells using the various viral probes can be found in Table 1. Enhanced
mutagenesis of a UV damaged probe was not as extensive as that
originally reported by Sarasin and Benoit (1980). These tabulated
results suggest that when observed, UVEM was small (less than 2) for
UV-irradiated viral probes in a variety of cell types (Table 1).
Untargeted mutagenesis was often enhanced in UV-irradiated cells and
appeared to be inducible with a requirement for de novo protein

synthesis (Table 1). This is similar to the inducibility of UVER and suggests that UVER may be linked to the untargeted response. Although UVEM has been characterized in a variety of mammalian cell types (Table 1), few studies have examined UVEM in normal or repair deficient human fibroblast strains. The possible contribution of error-prone repair processes to human carcinogenesis was therefore examined by comparing the UVEM of UV-irradiated adenovirus in normal and repair deficient (cancer-prone) human fibroblasts.

DNA Damage and Biological Expression of Ad2 following Gamma-Irradiation under Indirect (0°C) and Direct (-75°C) Conditions

Ionizing radiation will inactivate viral functions in a dose dependent manner. This has been shown for bacteriophage (Watson, 1950; Freifelder, 1965; 1966; Taylor and Ginoza, 1967; Boyce and Tepper, 1968; Van der Schans and Blok, 1970; Johansen et al, 1971; Coquerelle and Hagen, 1972; Clarkson and Dewey, 1973; Johansen et al, 1974) as well as animal viruses such as herpes virus (Lytle and Goddard, 1979), adenovirus (Rainbow and Mak, 1972) and poliovirus (Ward, 1980). Survival of virus following ionizing radiation depends a great deal on the composition and physical state of the surrounding media during the irradiation process. A predominance of direct radiation effects can be produced under conditions where the virus is irradiated in 1) a dehydrated condition; 2) a frozen state or 3) a liquid state but in the presence of high concentrations of protective organic compounds (Watson, 1952; Freifelder, 1965, 1966; Ginoza, 1968; Van der Schans and Blok, 1970). Indirect radiation effects predominate

in a dilute aqueous condition where free radicals and other toxic water products are formed and allowed to react with viral nucleic acid and protein.

The doses of radiation required to inactivate mammalian virus are much greater under direct than under indirect conditions. For example, D_o values for poliovirus increased from 14 Krads to about 200 Krads (or 140 to 2000 Gray where 1 Gy = 100 rad) with the addition of increasing concentrations of broth to the irradiation medium (Ward, 1980). Similarly, the inactivation of herpes simplex virus by X-irradiation in the liquid state was essentially exponential with a D_o of 43 Krads (Lytle and Goddard, (1979). Whereas, for X-irradiation in the frozen state, where direct effects predominate, a higher D_o value of about 150 Krads was obtained (Lytle et al, 1972).

It has been found for bacteriophage that one cause of biological inactivation under indirect conditions was due to protein damage which resulted in either loss of adsorption or release of DNA from the phage head into solution (Watson, 1952; Freifelder, 1965; Clarkson and Dewey, 1973). Capsid damage and subsequent release of the phage DNA into solution facilitated DNA degradation by radiolytic water products. Protein damage was found to be the main cause of inactivation of the double stranded RNA poliovirus, where 3/4 of the inactivation under indirect conditions was from loss of cell attachment due to protein damage (Ward, 1980). The remaining 1/4 of the inactivation was the result of damage to the viral genome. In contrast, the main cause of viral inactivation under direct conditions is due to damage of the viral nucleic acid (Rainbow and Mak, 1972;

Ward, 1980). Possible candidates for radiation induced nucleic acid damage which leads to inactivation of mammalian virus include strand breakage, cross-linkage, base damage and sugar damage.

Several studies have been carried out with gamma-irradiated virus that has been kept frozen at -75°C during irradiation. In one study, radiation induced DNA strand breakage was correlated with inactivation of biological functions of the virus (Rainbow and Mak, 1972). In other studies the HCR and ER of gamma-irradiated Ad2 was examined in normal and repair deficient human fibroblasts such as those from xeroderma pigmentosum (XP) patients (Rainbow and Howes, 1979; Jeeves and Rainbow, 1979). These studies showed that survival of Vag for gamma-irradiated Ad2 was significantly reduced in XP as compared to normal human fibroblast strains (Rainbow and Howes, 1979). The D_o value for the survival of Vag expression of gamma-irradiated Ad2 in XP25RO (Complementation Group A) was about 65% of that obtained in normal strains (Rainbow and Howes, 1979). Thus, gamma-irradiation at -75°C resulted in S.S. and D.S. breaks (Rainbow and Mak, 1972) as well as, or including DNA lesions which were poorly repaired in XP as compared to normal cells. Gamma-irradiation of adenovirus under frozen (-75°C) conditions may induce a small amount of "UV-like" DNA damage resulting in the reduced HCR observed in XP as compared to normal fibroblasts. In order to examine the biological and molecular effects of gamma irradiation under environmental conditions where indirect effects predominate, Ad2 was gamma irradiated at 0°C and assayed for dose-dependent loss of adsorption, plaquing and V-antigen production in KB and human fibroblast cells. This was correlated with molecular DNA damage in the form of SS and DS breaks.

TABLE I

ERROR-PRONE REPAIR OF UNDAMAGED AND UV-DAMAGED MAMMALIAN VIRAL PROBES IN HOST
CELLS INDUCED WITH DNA DAMAGING AGENTS

VIRUS	CELLS	INDUCING AGENT	MOI OF VIRAL PROBE (pfu/cell)	ER	EM	UNTARGETED INCREASE	TARGETED INCREASE	DELAY BETWEEN INDUCTION AND INFECTION	INHIBITION BY PROTEIN SYN. INHIBITORS	REFERENCE
H-1	MB-E (human)	UV, UV'd H-1,	10^{-2} for undamaged virus, 10 for UV'd virus	N.D.	N.D.	1.8-2.1	N.D.	t.c. (14 hrs)*	Yes	Cornelis et al, 1981
	RLSE (rat)	UV'd SV40		N.D.	N.D.	1.0-2.0	N.D.	t.c. (14 hrs)*	Yes	Su et al, 1981
H-1	MB-E RLSE	2-nitronaphtho-furans	10^{-3} (undamaged 5 (UV'd virus)	-1.0-2.3	N.D.					
H-1	MB-E RLSE	UV'd SV40, UV	10^{-3} (undamaged 5 (UV'd virus)	2.0, 2.45	<1	1.9-3.9	1.6	14 hrs	N.D.	Cornelis et al, 1982
H-1	MB-E	UV	0.002-1000	1.0-1.6	-1.0	1.6-1.8	N.D.	14 hrs	N.D.	Cornelis et al, 1982
H-1	RLSE, MB-E	UV'd { SV40 DNA, ΦX174 DNA, calf thymus DNA	10^{-3}, 0.1, 10	N.D.	N.D.	1.5-3.0	N.D.	20 hrs	N.D.	Dinsart et al, 1984
SV40	CV-1 (monkey)	UV	10^{-2} (undamaged virus) 10^{-4} (UV'd virus)	2.2-32	N.D.	N.D.	1.5-100	24 hrs	N.D.	Sarasin and Benoit, 1980
SV40	CV-1	acetoxy-acetyl-aminofluorene	1 (undamaged virus) 2×10^{-3} (UV'd virus)	N.D.	-3.0	-1	20	24 hrs	N.D.	Mezzina et al, 1981
SV40 (DNA)	CV-1	UV, mitomycin-C	? transfected	1.1-4.9, 1.4-3.0	N.D., -?	N.D., N.D.	-1.0, -1.0	24 hrs, 24 hrs	N.D., N.D.	Gentil et al, 1982
SV40	BSC-1 (monkey)	UV, UV	2.5×10^{-3}	1.04	0.34	3.2	1.1	t.c. (72 hrs)* 72 hr	N.D., N.D.	Cornelis et al, 1980
SV40	BSC-1	UV, UV	6×10^{-3}	0.12,0.97, 0.99	1.04, 0.55, 0.30	0.6-8.4, 0.9,1.8, 3.3.	-1.0	t.c. (72 hrs)* t.c. (24 hrs,UVEM) (101 hrs,UVER) (101 hrs, untargeted)	N.D.	Cornelis et al, 1981
SV40	CV-1	UV	-5×10^{-5}	2.6-7.6	0.302,0.019	1.7, 15	0.3, 0.5	24 hrs	N.D.	Taylor et al, 1982

* time delay for maximum mutagenic effects due to delay between cellular induction and viral infection

N.D. = not done

t.c. = time course

TABLE 1 (Continued)

VIRUS	CELLS	INDUCING AGENT	MOI OF VIRAL PROBE (pfu/cell)	ER	EM	UNTARGETED INCREASE	TARGETED INCREASE	DELAY BETWEEN INDUCTION AND INFECTION	INHIBITION BY PROTEIN SYN. INHIBITORS	REFERENCE
Ad5	CRL1187 A498 (human)	UV	~1.0	slightly >1.0	~1	0.02, 2.0	~1	24 hrs.	N.D.	Day and Ziolkowski, 1981
Ad2	HeLa	UV	1.0	3.0	<1.0	~1.5	~1	18 hrs.	N.D.	Piperakis and McLennan, 1984
Ad5	Normal human fibroblasts	UV	~0.4 (undamaged)	3.4	1.4	1.6	1.9	24 hrs.	N.D.	This Study
	Cockayne's Syndrome	UV	~1.0 (damaged)	1.6	1.1	1.3	1.5	24 hrs.	N.D.	This Study
		UV	~1.0 (damaged)	2.5	0.31	1.7	0.60	24 hrs.	N.D.	This Study
	Ataxia teleglectasia	UV	~1.0 (damaged)	1.4	0.73	2.0	1.3	24 hrs.	N.D.	This Study
	XP (group A) XP (variant)	UV UV	~1.0 (damaged)	2.1	0.52	1.6	0.80	24 hrs.	N.D.	This Study
HSV-1	Vero (monkey)	UV	1.0	1.5-3.8	0.73-1.2	1.0-2.3	0.73, 2.7	t.c. (16 hrs)*	N.D.	Das Gupta and Summers, 1978
HSV-1	CV-1	UV	0.2, 2.0	4.8, 2.5	0.70, 0.95	1.4	0.98, 1.3	24 hrs	N.D.	Lytle et al. 1980
HSV-1	MB-E	UV	0.2	~1.2-4.0	~1.8, 1.8, 2.2	~1	1.8-2.2	24 hrs	N.D.	Lytle and Knott, 1982
HSV-1	CV-1	UV	~1.0	N.D.	~1.0	~1	~1	20 hrs	N.D.	Takimoto, 1983
HSV-1	Normal	UV	~1.0	1.5-1.8	N.D.	2-4	N.D.	t.c. (24-48 hrs)	N.D.	Abrahams et al. 1984
	XPA	UV	~1.0	3.0	N.D.	3.0	N.D.	t.c. (24 hrs)	N.D.	Abrahams et al. 1984
	XPC	UV	~1.0	2.2	N.D.	2.0	N.D.	t.c. (48 hrs)	N.D.	Abrahams et al. 1984
	XPD	UV	~1.0	2.0	N.D.	3.0	N.D.	t.c. (48 hrs)	N.D.	Abrahams et al. 1984
	XP Variant	UV	~1.0	2.7	N.D.	3	N.D.	t.c. (72 hrs)	N.D.	Abrahams et al. 1984

* time delay for maximum mutagenic effects due to delay between cellular induction and viral infection

N.D. = not done

t.c. = time course

MATERIALS AND METHODS

Media

Media was purchased in powdered form from Grand Island Biological Company (GIBCO), New York.

a) Minimal Essential Medium (F-11, GIBCO catalogue # 410-1100) was used for monolayer culture of HeLa and KB cells.

b) Jocklik's Modified MEM (GIBCO catalogue # 410-1300) was used for Spinner (suspension) culture.

c) Alpha-MEM medium (GIBCO catalogue # 410-2000) was used for monolayer culturing of all human diploid fibroblast cell strains.

All media were prepared from powder according to the manufacturers instruction in double distilled water. The dissolved media were then filter sterilized through a Millipore filter (0.22 um) and stored at 4°C until use.

Solutions

a) Trypsin (0.25%) was purchased from GIBCO as a 1x solution without Ca^{2+} or Mg^{2+} (GIBCO # 610.5050). This was stored at -20°C and warmed to 37°C just before the subculturing of human cells. Unused solution was refrozen at -20°C before the next subculture.

b) Sodium bicarbonate ($NaHCO_3$) solutions (7 1/2%, W/V) were sterilized by autoclaving in 100 ml volumes at 15 lbs pressure for 15 minutes. These were stored at 4°C and used to adjust the pH of media preparations.

c) An Antibiotic-Antimycotic solution (100x) containing 10,000 U

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Penicillin (base), 10,000 mcg streptomycin (base) and 25 mcg Fungizone/ml in normal saline was stored at -20°C until use in media preparation. Unused solution was stored at 4°C (GIBCO # 600-5240).

d) BME Vitamin solution (100x) was stored at -20°C and warmed to 37°C before use as a media component. Unused solution was stored at 4°C (GIBCO # 320-1040).

e) BME Amino acids solution (100x) without L-glutamine was stored at 4°C before use as a media component (GIBCO # 320-1051).

f) Neutral red solution, 3333 mg Neutral red (sodium salt)/L distilled water and membrane filtered (GIBCO # 630-5330) was used as a vital stain in plaquing experiments.

g) L-arginine solution was prepared by dissolving 2.1% (W/V) L-arginine (Sigma, A-5006) in double distilled water followed by membrane filter sterilization (Millipore, 0.22 um). The solution was stored at 4°C until used in plaquing experiments.

h) L-glutamine solution (20 mM) was prepared by filter sterilization of crystalline L-glutamine (Sigma, G-3126) dissolved in double distilled water. Solutions were stored at 4°C until used in plaquing experiments.

i) Phosphate Buffered Saline was made up as a 10x concentrate as follows: 80 gm NaCl, 20 gm KCl, 11.5 gm Na_2HPO_4 and 2.0 gm KH_2PO_4 in 1 litre of double distilled water. This was diluted 1:10 with double distilled water to produce 1x PBS (without Ca^{++} or Mg^{++}).

j) TRIS buffered saline (TBS) was made in a 5x concentrated stock solution containing 80 gm NaCl, 3.8 gm KCl, 1.0 gm Na_2HPO_4, 300 ml of 1M Tris buffer (TRIZMA) pH 7.4 and 10 gm glucose dissolved in 2 litres

of double distilled water. This was sterilized by filtration through a .22 um Millipore filter and stored at 4°C until dilution with sterile distilled water to a 1x working solution. Purified virus was stored in TBS plus 20% glycerol at -75°C.

k) The conjugate used for fluorescent antibody staining was lyophilized sheep anti-rabbit globulin conjugated with fluorescein isothiocyanate (FITC) (GIBCO # 660-3512). This was rehydrated with 5 ml of double distilled water and subsequently diluted 1:20 with 1x PBS for use in staining infected fibroblast monolayers. Unused conjugate was stored at 4°C between stainings.

l) Alkaline lysing solution was prepared by dissolving 1.46 gm EDTA, 4.0 gm NaOH and 0.2 gm Sodium dodecyl sulphate (SDS) to 200 ml of double distilled H_2O. This was used to dissociate SS DNA from the protein components of virus samples.

m) Neutral buffer solution was prepared by dissolving NaCl (0.15M), sodium citrate (0.015M), TRIS HCl (0.010M), EDTA (0.005M), Sodium trichloroacetate (0.3M) in double distilled water and adjusting the solution to a final pH of 7.3.

Cell Lines

Human KB Cells

The human KB cell line originated from a human epithelial cheek carcinoma cell line and was originally obtained for this study from the laboratory of Dr. S. Mak, Dept. of Biology, McMaster. This cell line was grown in monolayer (on glass) or in spinner culture for viral production.

Human HeLa Cells

HeLa cells were originally obtained from a cervical carcinoma
and represent the first aneuploid epithelial-like cells of human origin
to be maintained continuously in tissue culture. The cells for this
study were originally obtained from the laboratory of Dr. F. Graham,
Dept. of Biology, McMaster University. These cells were grown in
monolayers (on plastic) as indicators of viral growth (plaquing) or to
produce adenovirus ts mutant stocks.

Vero Cells

The Vero cell line was initiated from the kidney of a normal
adult African green monkey and the cells were used to produce and titre
HSV-1 stocks. The cells were obtained from the lab of Dr. L. Prevec,
Dept. of Biology, McMaster University.

Human Diploid Fibroblasts

Normal Fibroblasts

Strain A2 was obtained from Dr. S. Goldstein, Departments of
Medicine and Biochemistry, McMaster University. Strain CRL1221 was
obtained from the American Type Culture Collection (ATCC, Rockville,
Maryland, USA). Strains GM969, GM288 and GM2674 were obtained from the
Human Genetic Mutant Cell Repository, Cambden, New Jersey, USA. The
human fibroblast strain Hff was obtained from Mr. J. Kawamotto, Dept.
of Microbiology, St. Joseph's Hospital, Hamilton, Ontario. These cells
were obtained from the foreskin of an apparently normal individual and

received in our laboratory at passage 5. All the strains were similarly obtained from apparently normal individuals.

Ataxia telangiectasia Fibroblasts

AT strain AT3BI was obtained from Dr. M. Paterson, Chalk River Nuclear Laboratories, Health Sciences Division, Chalk River, Ontario. AT homozygous strains GM1588 and GM3395 were obtained from the Human Mutant Cell Repository, Cambden, New Jersey.

Xeroderma Pigmentosum Fibroblasts

XP25RO (GM710) was obtained from the Human Mutant Cell Repository, Cambden, New Jersey.and belongs to XP complementation group A. CRL1162 was obtained from ATCC and has been classified as an XP variant cell strain.

Cockayne's Syndrome Fibroblast

CS strain GM2838 was obtained from the Human Mutant Cell Repository, Cambden, New Jersey.

Human KB Cell Line: Growth and Passaging

b). Monolayer cultures of KB cells were grown on glass in F11 supplemented with 10% (v/v) calf or newborn calf serum (GIBCO), 1% antimycotic/antibiotic solution and 0.75% (v/v) sodium bicarbonate solution. Confluent monolayers were resuspended by scraping the cells from the layer and passaged to fresh medium in a 1:3 split. KB cells used in plaquing experiments were resuspended and seeded into 60

mm plastic dishes (Nunc, A/S Nunc, Kamstrupvej 90, Kamstrap, DK4000

Roskilde, Denmark) such that 1 confluent Brockway would seed 10 60 mm

Nunc dishes. Cells were grown at 37°C in a humid incubation (90 - 100%

humidity) in a 5% CO_2 : 95% air atmosphere. KB cells were typically

confluent in 3 days after a 1:3 split under the growth conditions

described above.

b) KB suspension cultures were started by taking confluent monolayer

cultures of KB cells and resuspending to a cell density of 2×10^5

cells/ml. Cells were grown in Jocklick's MEM supplimented with 5%

(v/v) horse serum and 1-2% antimycotic-antibiotic solution. Cells

were kept in suspension using a magnetic stirrer and teflon coated

stirring bar. Cells were grown at 37°C.in a closed container stoppered

with a sterile cotton vent. Typically the spinner was "topped" with a

2-fold dilution of fresh Jocklick medium until the desired number of

cells was obtained.

Human HeLa Cells: Growth and Passaging

Monolayers of HeLa cells were grown on the surface of 150 mm

Nunc plastic plates in alpha-MEM supplemented with 10% (v/v) calf or

newborn calf serum (GIBCO), 1% (v/v) concentration of

antimycotic-antibiotic solution and 1% (v/v) sodium bicarbonate

solution. Confluent monolayers were washed 1x with 5 ml of alpha-MEM

(without serum). The wash was removed and the monolayers were

incubated at room temperature with fresh trypsin solution. Cells

detached from the plate were subcultured (1:3 split) to 150 mm dishes

containing complete alpha-MEM. HeLa cells used in plaquing experiments

were seeded into 60 mm (Nunc) plastic dishes such that each dish received 5 ml of HeLa cells suspended in complete alpha-MEM. Each confluent 150 mm dish was capable of seeding 10 60 mm plates which would be confluent 24 hours later when incubated at 37°C in a 5% CO_2 : air atmosphere.

Fibroblasts: Growth and Passaging

Diploid human fibroblast strains were grown in alpha-MEM supplemented with 10% (v/v) fetal calf serum (GIBCO), 1% (v/v) antimycotic/antibiotic solution, and 1% (v/v) sodium bicarbonate solution. Cells were grown as monolayers in 75 cm^2 plastic screwcap flasks (Falcon or Nunc plastic). Upon confluency the fibroblasts were passed by removing the old growth medium and washing the monolayers (1x) with 5-10 ml of alpha-MEM (without serum). Cells were detached by adding 3 ml of prewarmed trypsin solution. The detached monolayers were evenly resuspended in an additional 3 ml of complete alpha-MEM and distributed in a 1:3 split to new bottles containing fresh alpha-MEM (with FCS). A confluent monolayer of fibroblasts was sufficient to seed four 60 mm dishes which would be confluent after 24 hours of incubation at 37°C in a 5% CO_2 atmosphere and 90% humidity. Fibroblasts that were split 1:3 were typically confluent 7-10 days after growth at 37°C.

Vero Cells: Growth and Passaging

Vero cells were grown on glass in F15 modified MEM (GIBCO # 410-1500) supplemented with 10% newborn calf serum (v/v) and 1% (v/v)

sodium bicarbonate solution. Confluent monolayers were washed 1x with straight F15 (without serum) and cells were suspended by incubating the monolayers with 3 ml of warmed trypsin solution for 5-10 minutes at room temperature. Cells were then split 1:3 into fresh complete F15 and grown at 37°C in a 5% CO_2 atmosphere at 90% humidity.

Virus Stock Preparations

a) Adenovirus 2: KB cells were grown in spinner culture to a total of approximately 10^9 cells (2 - 3 litre volume) in Jocklick's medium. The cells were pelleted using low speed centrifugation in an IEC RP-2 centrifuge. The pellet was resuspended to give a final cell concentration of 10^8 cells/ml. The resuspended cells were infected with Ad2 at a multiplicity of 50-100 pfu/cell for 90 minutes at 37° on a roller wheel. After adsorption the cells were added back to the preconditioned spinner medium and topped with fresh complete Jocklick's to bring the concentration of cells to 3×10^5 cells/ml. The virus was grown for 48 hours at 37°C as a spinner culture after which the cells were pelleted by low speed centrifugation and resuspended in 0.01 M Tris buffer (pH 8.1) and frozen at -45°C until purification. The pellet was freeze-thawed 3x and sonicated on ice for 1-2 minutes using a Biosonic III, Model BPIII 40T (Bronwill Scientific, Rochester, New York), and subsequently, sodium deoxycholate was added to a final concentration of 0.5% (w/v) and incubated at room temperature for 30 minutes. The suspension was then adjusted to 2mM $MgCl_2$ and both deoxyribonuclease (Sigma) and ribonuclease (Sigma) were added to a concentration of 2 ug/ml. This solution was incubated at 37°C for

about 45 minutes and the solution adjusted to a density of 1.34 gm/ml by adding filtered saturated cesium chloride solution. This was centrifuged at 35K in a fixed angle Beckman T165 rotor using a Beckman model L2-65B ultracentrifuge. The virus band was collected and subjected to another cycle of isopycnic banding (20-24 hours, at 4°C) in CsCl (1.34 g/ml) and the resulting band was aseptically collected by drop fractionation. The concentration of the virus was determined by the absorbance of 1:10 and 1:20 dilutions of the virus in Tris buffered saline at 260 nm, with one 0.D. approximately equal to 2.5×10^{11} particles per ml. The virus suspension was diluted in TBS and 20% (v/v) glycerol such that stock virus generally contained $1-2 \times 10^{12}$ particles/ml which was stored at -70°C.

b) Adenovirus 5ts36 was obtained from N. Lassam and was prepared in a manner identical to that for Adenovirus 2 except that KB cells were infected and grown at 33°C for 96 hours in suspension culture before viral harvest. Sonication was performed as described for Ad2 after which an equal volume of Freon 113 (Matheson) was added and the mixture was homogenized for 1 minute in a Sorval omnimixer (setting 7.5). the homogenate was centrifuged for 2 minutes at 1,000 x g in an IEC centrifuge after which the virus containing supernatant was removed and placed in a cold 250 ml graduated cyclinder. The remaining Freon phase was re-extracted twice with 20 ml of 0.1M Tris (pH 8.1) and the supernatants were pooled, poured into a buret and dripped into Beckman ultracentrifugue tubes containing 7 ml of CsCl, Tris buffer (0.1M Tris, pH 8.1, 1.43 gm/ml CsCl). This was centrifuged in a Beckman SW-27 rotor at 50,000 xg for 90 minutes. After centrifugation, the

supernatant was removed by aspiration, leaving an opalescent virus band

on top of the CsCl cushion. This material was collected using a

pipette and adjusted to a density of 1.34 using saturated Cscl

solution. This was then centrifuged as described for the preparation

of Ad2. The purified virus was adjusted to a density of 1×10^{12}

particles/ml and stored at -70°C as described for Ad2. This viral

stock was checked for plaquing ability on confluent HeLa monolayers at

33° (permissive) and 39° (nonpermissive) temperatures resulting in a

reversion of frequency of 5.9×10^{-5}.

c) Ad5t36 was alternatively prepared by diluting (10 fold) a 0.1,ml

stock of Ad5t36 (with a titre of 7.6×10^8 pfu/ml at 33°C as assayed on

KB cells) with 0.9 ml of alpha-MEM (without serum). This stock

produced a reversion frequency of 1.3×10^{-5} as assayed on HeLa cells.

A portion (0.2 mls) of this diluted stock was infected onto 60 mm

confluent HeLa cell monolayers (90 minutes at room temperature) and

unadsorbed virus were removed by washing (1x) with straight alpha-MEM.

Virus was grown at 33°C for 96 hours in complete alpha-MEM (3 ml/60 mm

dish). These infected cells were collected by scraping into the 3 ml

of growth medium and virus were released by repeated (3x) cycles of

freeze thawing at -20°C and 37°C. These viral stocks were then pooled

cellular debris was removed by low speed centrifugation and 3 ml

aliquots of this pooled stock were infected onto 150 mm confluent

monolayers of HeLa cells at room temperature for 90 minutes. The

unadsorbed virus was removed by washing (1x) with "straight" alpha-MEM.

The monolayers were refed with 10 ml of complete alpha-MEM and allowed

to grow for 96 hours at 33°C in a humid CO_2 (5%) atmosphere. Cells

were collected and virus were released by freeze-thawing as described above. Virus recovered from the supernatant had a reversion frequency of 6.3×10^{-5} as assayed on HeLa cell monolayers.

d) Ad5ts125: a sonicated crude cell extract of Ad5t125 was obtained from Dr. F.L. Graham, Dept. of Biology, McMaster University. 0.3 ml of this crude stock was diluted with 2.7 ml of "straight" F11 MEM. This stock was infected (1 ml) onto confluent 150 mm monolayers of KB cells for 90 minutes at room temperature. Unadsorbed virus was removed by washing (1x) with "straight" F11 MEM. The infected cells were grown for 96 hours at 33°C in 15 ml of complete F11 (with 10% calf serum). Cells were scraped into the growth medium and virus were released by alternate cycles of freeze thawing (3x) as described above. This stock of Ad5ts125 had a reversion frequency of 6.8×10^{-5}.

e) Herpes Simplex virus (HSV-1 KOS): A stock of HSV-1 was obtained from the lab of Dr. L. Prevec, Dept. of Biology, McMaster University. This stock had a titre of 2×10^8 pfu/ml as assayed on Vero cell monolayers. 0.2 ml of this stock was diluted in 0.8 ml of straight alpha-MEM. Aliquots of this diluted stock (0.3 ml) were infected onto 3 confluent monolayers of Hff fibroblasts contained on Brockway bottles. After adsorption for 90 minutes, unadsorbed virus was removed by washing with "straight" alpha-MEM. Each Brockway was refed with 10 ml of complete alpha-MEM. Extensive CPE was observed after 24 hours of growth at 37°C in a 5% CO_2 atmosphere. Cells were collected by scraping into the growth medium at 48 hours post infection and virus was released by alternate cycles of freeze-thawing (3x).

Preparation of Radiolabelled Ad2

Radiolabelled virus was prepared in a similar manner as for unlabelled Ad2, except that the virus was labelled with ^{3}H-thymidine in its DNA using the following procedure: At 9 hours post infection, 1 mCi of ^{3}H-thymidine (specific activity 20 Ci/mM) together with 150 ugm of cold (unlabelled) thymidine was added to each litre of infected spinner culture. The infected culture was incubated a further. 39 hours at 37°C at which time the labelled virus was purified as described previously. In the case of ^{14}C labelled virus, 50 uC; of ^{14}C-thymidine was added to the infected culture, without the addition of cold thymidine. Viral growth and purification was similar to that described for ^{3}H labelled virus.

UV-irradiation of Virus

All adenovirus stocks were diluted 2-fold with "straight" alpha-MEM and placed in either 35 or 60 mm petri dishes (Falcon plastic) before UV-irradiation. Virus was kept on ice, with constant swirling during UV-irradiation at a dose rate of 6.6 J/m^2. UV-irradiations were performed in an open, depth adjustable box illuminated with a germicidal lamp (General Eletctric, G8T). Incident dose rates were determined using a J-225 short wave UV meter (Ultraviolet Products, San Gabriel, California).

Gamma-irradiation of Ad2 at -75°C

Gamma-irradiation of Ad2 at -75°C was performed using a ^{60}Co source of about 5 KCi as described by Jeeves and Rainbow, 1979. Two ml

of stock Ad2 were kept on dry ice (-75°C) during irradiation in order to minimize thermal inactivation of viral infectivity. Under these conditions, the dose rate was about 1 Mrad per hour, as determined by a standard Fricke chemical dosimeter.

Gamma-irradiation of Ad2 at 0°C

Gamma irradiation of Ad2 in the liquid state was carried out at 0°C (on ice) using a ^{137}Cs source and a perspex sample holder as previously described (Rainbow and Mak, 1972). Virus was irradiated in a liquid state suspended in TBS plus 20% glycerol at a dose rate of approximately 19 Krad/hour.

UV-irradiation of Cell Monolayers

Monolayers of human fibroblast strains were UV-irradiated in either 8 well chamber slides (Lab Tek) or 60 mm plastic petri dishes (Nunc). Growth medium was removed and the monolayers were immediately UV-irradiated at room temperature at a dose rate of 1 J/m^2sec. The only exception was that the cell strain XP25RO was UV-irradiated at the maximum depth of the UV box such that a total of either 0.2 or 0.4 J/m^2 was delivered to the monolayers. The UV lamp and UV meter were similar to that described for the UV-irradiation of virus.

Plaque Assays

Throughout this study, plaque assays of Ad2 and Ad5ts mutants were carried out on human KB or HeLa cell lines and on human fibroblast strains. These assays were performed at 33°, 37° or 39°C, depending

on the experimental conditions. The procedure varied .slightly depending on the cell monolayers used for the assay.

a) <u>Human KB or HeLa cell monolayers</u> Cells were grown at 37°C in 60 mm sterile petri dishes (Nunc) until 80-100% confluent. The growth medium was removed and the monolayers .were infected with 0.2 ml of various viral suspensions diluted in "straight" alpha-MEM. Virus was adsorbed for 90 minutes at room temperature with frequent rocking of the monolayers to evenly. distribute the virus. After adsorption of the virus, each monolayer was overlaid with 10 ml of nutrient agar medium (held at 43°C) and prepared by mixing equal volumes of solutions A and B (below) just before overlaying the monolayers.

<u>Plaquing Solution A:</u> prepared aseptically as follows using the volumes indicated.

F11 (2x) Eagles MEM,
GIBCO # 410-1100   320 ml

BME Amino Acids (100x),
GIBCo # 320-1051   10 ml

Vitamin Solution (100x),
GIBCO # 320-1040   10 ml

Newborn Calf Serum*,
GIBCO # 200-6010   60 ml

Horse Serum,
GIBCO # 200-6650   60 ml

* Note: Calf serum (GIBCO # 200-6170) was substituted for Newborn
        calf serum in.some instances.

Antimycotic-Antiobiotic solution 100x,
GIBCO # 600-5240   10 ml

L-glutamine (2 mM)   8 ml
Sigma

L-arginine (2.1%)   6.5 ml
Sigma

Sodium Bicarbonate (7.5%)   35 ml
Fisher Scientific

Plaquing Solution B: autoclaved at 15 lbs for 15 minutes

double distilled water   .   200 ml

Purified Agar
Difco # 0560-01   3.6 gm

$MgCl_2 6H_2O$
Mallinckrodt # 5958   1.6 gm

The monolayers were incubated for 5, 8 or 12 days at 39°, 37°,
or 33°C respectively, depending on the virus stocks used. Plates were
then overlaid with 5 ml of plaquing medium containing 1% (v/v) Neutral
red (GIBCO # 630-5330). For the neutral red overlay, Bacto-agar (Difco
0140-01, 1% w/v) was substituted for the purified agar used in
plaquing solution B. Clear plaques were visible 2 days after neutral
red overlay and scored every other day until counts stabilized. Plaque
counts at 33°C generally stabilized 20-25 days post viral infection,
plaque counts at 37°C generally stabilized 15-20 days after infection
while plaque counts obtained at 39°C, stabilized 10 to 15 days after
viral infection.

b) Human fibroblast strains: Plaque assays were very similar to the
procedure described for KB or HeLa cells. Plaquing was performed at
37° or 33°C with the same plaquing solutions A and B. The only change

was a substitution of fetal calf serum for newborn calf serum in Solution A. Neutral red overlay was at 12 days post infection (37°C) or at 15 days post-infection (33°C). Plaques were counted until constant numbers were observed.

Adenovirus Progeny Assay

Confluent human fibroblast stains, contained in 75 cm^2 plastic flasks, were typically split into four 60 mm petri dishes and grown until confluent at 37°C for 24 hours in 5 ml of complete alpha-MEM (10% FCS).

Growth medium was aseptically removed and two plates were UV-irradiated at a dose rate of 1 $J/m^2/sec$. The plates were either:

a) Immediately infected with intact (unirradiated) or UV-irradiated adenovirus ts mutant stock such that 0. 2 ml of a 10^{-2} stock dilution (in "straight" alpha-MEM) of intact virus was infected onto both a control monolayer and a UV-irradiated monolayer. Similarly a 0.2 ml dilution of UV-irradiated virus (a 1 ↑ 1 dilution of stock: "straight" alpha-MEM) was infected onto a control monolayer and a UV-irradiated monolayer. Virus was allowed to adsorb for 90 minutes at room temperature, then washed (1x) with 5 ml of "straight" alpha-MEM per monolayer. The wash was aseptically removed and 3 ml of complete growth medium was added to each monolayer. These infected cells were incubated for 96 hours after which the fibroblasts were resuspended by scraping them into the growth medium. Infected cell suspensions were immediately frozen at -20°C.

b) Fibroblasts were often refed with 5 ml of complete alpha-MEM to

measure UV-inducible responses associated with delayed viral infection. Viral infections (as described) were delayed 12, 24, 36 or 48 hours after cellular UV-irradiation. Fibroblasts were grown at 37°C during the UV-delay periods.

Viral progeny from intact and UV-irradiated virus infecting either unirradiated or UV-irradiated fibroblasts were released by 3 cycles of freeze-thawing. After the last thawing the tubes were thoroughly mixed and cellular debris was allowed to settle. Viral progeny (0.1 ml) were obtained from the supernate and passed through serial 10 fold s dilutions in "straight" alpha-MEM. Aliquots (0.2 ml) of progeny dilutions were infected onto confluent KB or HeLa monolayers in quadruplicate and plaque counts were determined at 33° and 39°.

Progeny Growth Kinetics

One step, single cycle Ad5ts36 growth curves were determined for intact and UV-irradiated virus grown on Hff monolayers. These curves were used to determine the end of a single lytic cycle of Ad5ts36 grown in normal human fibroblasts at 33°C.

The UV-irradiation and infection protocol was as described above. Progeny from intact and UV-irradiated virus grown in unirradiated and UV-irradiated Hff cells was collected at 12, 24, 36, 48, 60, 72, 84 and 96 hours post viral infection. Titres of these progeny were determined on KB cells at 33°C as described above.

UV-induced Reversion Kinetics of Ad5ts36 and Ad5ts125

The kinetics of UV-induced reversion and survival of the

adenovirus mutant progeny were determined in normal human fibroblast strain CRL1221. The progeny assay was as described above with the exception of viral UV-irradiation. Viral stock (1.5 mls) was diluted with "straight" alpha-MEM (1.5 mls) and placed in a sterile 100 mm petri dish (Corning). Virus was UV-irradiated (on ice) at 6.6 $J/m^2/sec$ and 0.2 ml aliquots were removed at 200 J/m^2 (to.virus) intervals until a total of 1,600 J/m^2 was given to the virus. Unirradiated (intact) virus was diluted to 10^{-2} and 0.2 ml aliquots were infected onto duplicate control (unirradiated) monolayers. Infected cells were grown for 96 hours at 33°C. The cells were harvested and viral progeny were assayed as described above. The titres obtained at 33°C were used to construct survival curves for both mutant viruses.

Induction of Ad5ts36 Reversion by Preinfection with Lethally UV-irradiated HSV-1 (KOS)

A HSV-1 (KOS) stock with a titre of 1 x 10^6 pfu/ml (as assayed upon Vero monolayers) was UV-irradiated at 1.2 $J/m^2/sec$ to a total dose of about 1,100 J/m^2. Unirradiated human fibroblasts were grown to confluency in 60 mm petri dishes. UV-irradiated HSV-1 was infected (0.2 ml of UV-irradiated stock) onto 3 fibroblast plates for 60 minutes at room temperature. The plates were washed 1x in "straight" alpha-MEM, then refed with 5 ml of complete alpha-MEM and allowed to incubate for 24 hours at 37°C. The untreated (without UV-irradiated HSV) and two of the monolayers preinfected with UV-irradiated HSV-1 were infected with intact and UV-irradiated Ad5ts36 as described above. The growth and harvesting of Ad5ts36 was as described previously. One

monolayer infected with only HSV-1 was harvested at the same time as the Ad infected monolayers, freeze-thawed in a similar manner and analysed for HSV progeny on HeLa monolayers.

Time Courses of the UVER/UVEM Expression Using Ad5ts36 and Ad5ts125 Probes in Human Fibroblast Strains

The induction of Ad5ts36 UVER/UVEM responses in normal fibroblast strains and CRL1162 was examined when viral infection was delayed 0, 12, 24 or 36 hours following UV-irradiation of the cell monolayers. Fibroblasts were seeded into 60 mm sterile petri dishes which were divided into groups of 4 plates per time point. Initially, (after reaching confluency) a group of 4 plates were aspirated and 2 of the plates received at total of 10 J/m^2. These cells were refed with 5 ml of complete alpha-MEM and placed back into the incubator at 37°C. Twelve hours later a second group of 4 plates had their growth medium removed, were UV-irradiated, refed and reincubated as above. This procedure was carried out for the 12 hour delay time point but at 0 hour (immediate viral infection), all plates were aspirated and infected with intact or UV-irradiated Ad5ts36 (1,200 J/m^2) such that each time point was infected and adsorbed (90 minutes at room temperature) with virus from the same UV-irradiated stock. Progeny growth and harvesting was identical to that described above. In a similar manner, Ad5ts125 was used to examine the time course expression of UVER/UVEM responses in human strains CRL1221.

Adenovirus Reactivation using the "V" antigen Assay

This assay has been used previously in the examination of host cell reactivation and enhanced viral reactivation phenomena (Rainbow, 1981; Jeeves and Rainbow, 1983a; b; c). Briefly, unirradiated or irradiated Ad2 suspensions were assayed for their ability to express "late" viral structural antigens ("V" antigen) in unirradiated (control) or irradiated human fibroblast monolayers. The procedure generally used was as follows:

1) One 8 well chamber slide (Lab Tek Products, Napierville, Ill.) was seeded with one confluent 75 cm^2 monolayer of human fibroblasts. Each confluent 75 cm^2 monolayer was washed 1x with "straight" alpha-MEM, then incubated for 15 minutes at room temperature in 3 ml of trypsin solution. Cell suspensions were diluted in complete alpha-MEM such that 0.4 ml of suspension was seeded per well. In experiments that required more than one slide, trypsinized cells were pooled before distributing into individual wells. The fibroblasts were grown to confluency such that each well contained approximately 4 x 10^4 cells.

2) The infections of intact and UV-irradiated virus were carried out on separate chamber slides such that three serial dilutions were used to infect duplicate wells on the same slide. Two additional wells per slide served as uninfected controls. The growth medium was removed by aspiration before viral infection with 25 ul of the appropriate dilution. Virus was adsorbed for 90 minutes at room temperature with frequent rocking of the slides. After viral adsorption, each well was refed with 0.4 ml of complete alpha-MEM and incubated at 37°C for 48

hours. The slides were aspirated to remove the growth medium and subsequently washed 3x with 1x PBS for 5 minutes per wash. The final PBS wash was removed and the fibroblasts were fixed by adding 0.3 ml of a cold (-20°C) acetone: methonol (1:1) solution. Cells were fixed for 10-15 minutes, drained of fixative and allowed to air dry. The plastic wells and rubber gaskets were removed and slides were stored at -20°C until staining.

Fluorescent Staining

An indirect fluorescent antibody staining method was used to detect adenovirus infected fibroblasts. Infected cells were first adsorbed with rabbit anti-Ad2 "V" ag antiserum followed by treatment with fluorescein isothrocyanate conjugated sheep anti-rabbit globulin using the following procedure.

Fixed slides were rehydrated with 1x PBS for 30 minutes at 37°C. A drop (20 - 30 ul) of a 1:23 dilution of stock rabbit anti-Ad2 serum in PBS was added to a number of 22 x 22 mm coverslips (Corning Glass Works, Corning, New York). Slides were drained of excess PBS and 2 coverslips were placed on each slide such that one coverslip covered 4 of the 8 cell sheets on a slide. Air bubbles were removed and the slides were incubated in an inverted position for one hour at 37°C. The slides were then washed 3x in PBS such that the final was 30 minutes at 37°C. A drop (20 - 30 ul) of a 1:20 dilution of stock FITC conjugated sheep anti-rabbit globulin in PBS was placed on a number of fresh coverslips. Slides were drained of excess PBS and 2 coverslips were placed over each slide's monolayers. Air bubbles were removed and

incubated at 37°C for one hour. Coverslips were removed in PBS and washed 3x such that the final wash was 30 minutes. A drop of PBS-glycerol (1:9) was placed on each of a fresh set of coverslips and applied to slides drained of excess PBS. Prepared stained slides were stored at 4°C until counting.

Fluorescent Microscopy

Stained monolayers were examined using a Leitz Orhoplam Fluorescent microscope (Leitz, Wetzlar, Germany). A 490 mm excitor filter was used in conjunction with a 510 mm barrier filter. Infected fibroblasts appeared bright green against a dark background which indicated the expression of adenovirus Vag. The number of Vag positive cells were counted for duplicate wells at 3 serial 2 fold dilutions of the virus. These counts were fitted to a straight line using regression analysis by the method of Daniel (1974) and the slope of the line was used as a quantitative measure of Vag formation.

Preparation of Ad2 Antiserum

Ad2 antigen was prepared for injection by mixing 0.4 ml (approximately 8×10^{11} particles) of stock virus, 1.6 ml of sterile TBS and 2 ml of Freunds complete adjuvant (GIBCO # 600-5721), and the suspension was made homogeneous by forcing the components between two syringes joined by a two way hypodermic needle. One ml of the mixed antigen was injected intramuscularly and one ml intraperitoneally, into each of two New Zealand white rabbits. At 20 and 36 days after the initial challenge, the rabbits were injected with 0.5 ml of a mixture

of 0.4 ml of virus stock and 1.6 ml of sterile TBS, both subcutaneously and intramuscularly. The animals were cannulated 8 days after the last injection. Blood was allowed to clot overnight at 4°C and the resulting immune serum (145 ml) was stored at -20°C in 10 ml aliquots.

Host Cell Reactivation of Gamma-irradiated Ad2

Both non-irradiated and gamma-irradiated suspension of Ad2 were assayed for their ability to form Vag in control (unirradiated) human fibroblast strains. Ad2 gamma-irradiated at 0° or 75°C, were infected onto confluent monolayers of fibroblasts in 8 well chamber slides as described above. Virus was adsorbed for 90 minutes at room temperature and were then circulated at 37°C for 48 hours in complete alpha-MEM growth medium. At 48 hours after infection, slides were fixed and stored as described previously.

Time Course of Vag Expression

Normal human fibroblast strain Hff was grown to confluency in 8-well chamber slides and were either left unirradiated or UV-irradiated to a total of 15 J/m^2. Fresh complete alpha-MEM was used to refeed all the monolayers and the fibroblasts were reincubated at 37°C for 24 hours, at which time the monolayers were infected with intact or UV-irradiated Ad5ts36 dilutions. The unirradiated virus was diluted such that 0.020 ml of stock was diluted into 4.0 ml "straight" alpha-MEM while UV-irradiated (1,200 J/m^2) virus was diluted (0.6 ml stock in 5.4 ml of "straight" MEM) before UV-irradiation. The stock was assayed as having approximately 1.9×10^6 vfu/ml as assayed

on normal human fibroblasts using rabbit anti-Ad2 antiserum as described above. Virus were allowed to absorb for 90 minutes, at which time the infected monolayers were incubated at 33°C with fresh alpha-MEM growth medium. The infected monolayers were fixed and stored at 12 hour intervals from 12 to 120 hours after infection. These slides were stained exactly as described for Ad2.

UV-enhanced Reactivation of Vag Expression in Human Fibroblast Strains

Normal and AT fibroblasts were grown to confluency on 8 well chamber slides. Growth medium was removed and monolayers were either UV-irradiated or left unirradiated, refed with growth medium (in the case of delayed infections) and incubated at 37°C. Dilutions of intact or UV-irradiated Ad2 were infected onto monolayers of fibroblasts such that infections were either at 0, 24, 36 or 48 hours following cellular UV-irradiation. Virus was grown at 37°C for 48 hours and fixed as described above. In the case of UVER experiments at 33°C, virus was fixed at 72 hours post infection. UVER experiments involved in the time course of Vag expression were UV-irradiated such that viral infections were concomitant, using the same intact and UV-irradiated viral stock dilution series.

Viral Adsorption

Each dose of gamma-irradiation to ^{3}H labelled Ad2 (10^7 cpm/ml corresponded to approximately 10^{12} particles/ml) was absorbed to 5×10^6 human KB cells at 37°C using a rolling shaker. Ninety minutes after virus addition, the cells were diluted 5x with warm growth medium

plus 10% FCS and incubated a further 30 minutes. The cells from the infected culture were pelleted at low speed and the radioactivity of the pellet determined after washing 3x in PBS. After washing, the pellet was resuspended to a volume of 0.5 ml in PBS and mixed with 10 ml of scintillant (Aquasol, New England Nuclear Company). This was then counted in a Beckman Liquid Scintillation spectrometer.

A.I. GROWTH KINETICS OF ADENOVIRUS ts36 PROGENY PRODUCTION IN

NORMAL HUMAN FIBROBLASTS AT THE PERMISSIVE TEMPERATURE

To determine if the production of UVER is accompanied by UVEM

for UV-irradiated adenovirus in human cells, it was necessary to

determine the experimental conditions required for the maximal

expression of UVER. The kinetics of viral growth at the permissive

(33°C) temperature was determined by harvesting viral progeny at

increasing time intervals from unirradiated or UV-irradiated human

foreskin fibroblasts (Hff) infected with either unirradiated or

UV-irradiated Ad5ts36. Infections were carried out either immediately

(Figure 1A,B,C) or delayed 24 hours (Figure 1D,E,F) after cellular

UV-irradiation. The titre of the Ad5ts36 stock (when plaqued on Hff at

33°C) was 3×10^8 pfu/ml.

Unirradiated virus was diluted in alpha-MEM (without serum) so

that 6×10^5 pfu's were added to UV-irradiated and unirradiated

confluent Hff monolayers in 60 mm dishes. UV-irradiated virus (1200

J/m^2) was used to infect Hff monolayers in 60 mm dishes such that a

total of 3×10^7 pfu's (before viral irradiation) was added to each

UV-irradiated and control Hff plate. In a separate experiment, a UV

dose of 1200 J/m^2 to the virus gave a surviving fraction of 5.3×10^{-2}

for this Ad5ts36 stock when plaqued on the normal fibroblast strain A2

at 33°C. This suggested that after a UV dose of 1200 J/m^2 to the

virus, the titre on Hff would decrease to about 1.6×10^6 pfu assuming

a similar level of host cell reactivation between these 2 normal cell

strains. Based on the above considerations the multiplicity of infection (m.o.i.) in Figure 1 was approximately 0.6 for unirradiated virus and 1.6 for UV-irradiated virus (there were about 10^6 fibroblasts per confluent 60 mm dish estimated by viable cell counts of resuspended cells). Viral progeny were released by freeze-thawing fibroblasts 3X after they were collected by detaching monolayers with sterile rubber policeman and the progeny titres were subsequently determined by plaquing at 33°C on KB cell monolayers.

Plaque titres upon KB cells typically stabilized after about 20 days incubation at 33°C. All titres were determined from 4-8 replicate plates which displayed from 30-300 plaques per 60 mm dish. Each countable dilution was preceeded and followed by a 10 fold dilution which was similarly plaqued on KB cells. It was therefore possible to check the accuracy of the 10 fold-dilution technique for each individual viral progeny titre. Any dilution series which did not display the characteristic 10 fold decrease upon each dilution was repeated. This served to minimize the inaccuracies due to dilution error.

Figure 1 shows the results obtained for progeny growth when infection was carried out immediately (within half an hour; Figure 1A,B,C) or 24 hours (Figure 1D,E,F) after cellular UV-irradiation. It can be seen that for both infection delays, the onset of production of viral progeny was detected earlier for unirradiated virus compared to that for UV-irradiated virus. The capacity to support viral growth was decreased for both unirradiated and UV-irradiated virus in UV-irradiated cells as compared to unirradiated fibroblasts when viral infections were assayed immediately after UV-irradiation (Figure 1A,B).

Figure 1

The single-cycle kinetics of viral progeny production following immediate or delayed infection of unirradiated or UV-irradiated human foreskin fibroblasts (Hff) with intact and UV-irradiated Ad5ts36.

Confluent Hff cells were UV-irradiated (closed symbols) immediately (panel A,B,C) or 24 hours prior to (panel D,E,F,) viral infection with unirradiated or UV-irradiated virus. Unirradiated Hff monolayers were infected in a similar manner. The moi was 0.6 pfu/cell for unirradiated virus and 1.6 surviving pfu/cell for UV-irradiated virus (as titred on non-irradiated human fibroblasts). Virus was adsorbed for 2 hours at room temperature and viral progeny collected after lytic growth at 33°C for the time periods indicated. Plaque titres of progeny were determined upon confluent KB cell monolayers at 33°C. Plaques were counted daily until the titre stabilized. The average standard errors associated with the plaque determinations were 5.4% (immediate infection) and 9.8% (24 hour delayed infection). These values are $\pm 1SE$ of each plaque titre expressed as a percentage and resulting error bars are contained within the data points.

TOP PANEL : unirradiated Ad5ts36

Panel A (immediate)   Panel D (24 hour delay)

O No UV dose to the   O No UV dose to the
   cells   cells

● 10 J/m^2 to the cells   ● 15 J/m^2 to the cell

CENTER PANEL: UV-irradiated virus (1200 J/m^2)

Panel B (immediate)   Panel E (24 hour delay)

△ No UV dose to the[+]   △ No UV dose to the[f]
   cells   cells

▲ 10 J/m^2 to the[*]   ▲ 15 J/m^2 to the[f]
   cells   cells

BOTTOM PANELS: UVER Factor

[+]No plaques observed 24-48 hours
[*]No plaques observed 24-60 hours
[f]No plaques observed 12-24 hours

Figure 1

75

IMMEDIATE INFECTION   24 HOUR DELAYED INFECTION

HOURS POST INFECTION
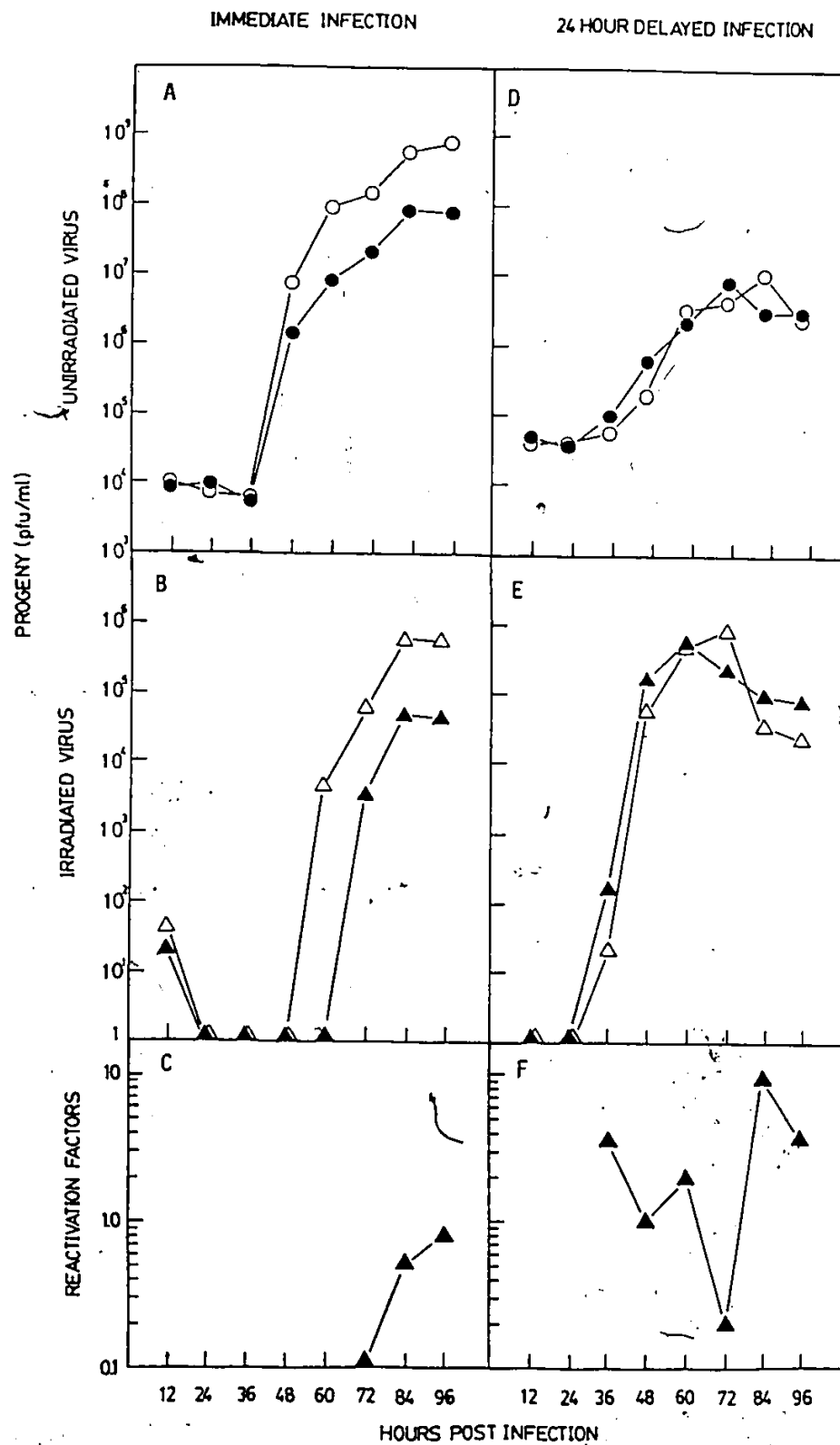
In cells infected 24 hours after cellular UV, an enhanced progeny production was observed in UV-irradiated as compared to unirradiated cells infected with UV-irradiated virus (Figure 1E). No difference was observed in the progeny production resulting from infections of an unirradiated probe in unirradiated as compared to UV-irradiated monolayers (Figure 1D) when infections were delayed 24 hours after cellular irradiation. Comparison of data derived under delayed or immediate infection protocols revealed changes in the growth kinetics (Figure 1). Most striking, is that no delay in the onset of viral progeny production was seen for UV-irradiated virus infecting irradiated as compared to unirradiated monolayers (using the delayed (Figure 1E) but not the immediate (Figure 1B) infection protocol. With both infection protocols (Figure 1) the cycle of virus replication appears to be complete by 96 hours for both unirradiated and UV-irradiated virus. Unirradiated virus grown in control cells produced a maximum progeny yield at 96 hours (1.1×10^9 pfu/ml) while undamaged virus grown in cells UV-irradiated immediately before infection (Figure 1A) had a maximum yield at 84 hours post infection (9.6×10^7 pfu/ml). The maximal yield of viral progeny using the 24 hour infection delay protocol was observed at 84 hours post infection for unirradiated virus infecting either unirradiated (1.1×10^7 pfu/ml) or UV-irradiated (7.7×10^6 pfu/ml) fibroblasts(Figure 1D). In both protocols viral progeny yields were greatest from unirradiated fibroblasts infected with unirradiated virus.

UVER of UV-irradiated adenovirus were calculated for the 72, 84 and 96 hour time points during the lytic cycle were 0.1, 0.52 and 0.79 respectively (immediate infection, Figure 1C). These values were

determined by calculating the ratio of the surviving fraction of viral progeny in UV-irradiated fibroblasts to the surviving fraction of viral progeny in non irradiated fibroblasts. UVER values greater than 1 indicate that cellular UV-irradiation has stimulated the reactivation (increased viral survival) of UV damaged virus while values less than 1 suggests that reactivation of UV-irradiated virus was inhibited due to irradiation of the host cells. The UVER obtained thus indicated that during this lytic cycle time course no UV enhanced reactivation was observed for progeny production in cells irradiated immediately prior to infection. UVER values can similarly be calculated for viral progeny titres obtained from 36 to 94 hours of the lytic cycle when viral infections were delayed 24 hours after cellular irradiation (Figure 1F). The largest UVER factors of 9.2 and 3.7 were found at the 84 and 96 hour time points at the end of the lytic cycle growth curve. This data suggests that a 24 hour delayed (but not immediate) viral infection facilitated the expression of UVER of UV-irradiated adenovirus progeny. Since a second lytic cycle was not observed after 96 hours of viral growth, subsequent progeny assays of normal or repair deficient human fibroblasts were infected 24 hours after cellular UV-irradiation and collected at 96 hours post infection.

As a result, in subsequent experiments both normal and repair deficient cells were infected with Ad5ts36 24 hours after UV cells and viral progeny were collected at 96 hours post-infection.

A.II.   The Kinetics of Ad5ts36 Viral ("V") antigen

Production in Hff Cells; Viral Infection Delayed 24

Hours Following UV (15 J/m^2) to the Cells

Previous work in our laboratory has emphasized the use of the "V" antigen technique to characterize UVER responses in both normal and repair deficient human fibroblasts (Jeeves and Rainbow 1983a; 1983b). It was therefore considered of interest to characterize the "V" antigen UVER response of Ad5ts36 in Hff' cells under similar experimental conditions (run concurrently) as the progeny growth curve described previously in Figure 1.

Hff cells were grown to confluency in 8-well chamber slides at 37°C. These cells were infected 24 hours after UV (15 J/m^2) to the cells. Infected cells monolayers were then incubated at 33°C and fixed at 12 hour intervals from 24 to 120 hours post infection.

Figure 2 shows the results of the time course of "V" antigen production following infection of confluent Hff cells with Ad5ts36. Differences observed between Figures 1 and 2 may be attributable to the different biological endpoints being assayed and/or to differences in the moi between the two experiments. The "V" antigen assay was conducted at lower moi such that (90-360) vfu of intact virus was infected onto monolayers (about 4 x 10^4 cells) per control or UV-irradiated well. UV irradiated virus was infected onto monolayers such that (40-170) surviving vfu of UV-irradiated virus was infected onto unirradiated or UV-irradiated monolayers.

Calculation of UVER values from 60 to 120 hours (Figure 2C) revealed that all were greater than 1 and peaked at 96 hours with a UVER value of 9.8. This indicated that UVER as measured by the

expression of "V" antigen positive cells was enhanced for UV-irradiated virus in cells UV-irradiated 24 hours prior to infection.

The UVER values determined from the "V" antigen assay were not significantly different from those found for the progeny assay and both techniques expressed UVER values greater than one. The data of Figure 2 is very similar to that published by Jeeves and Rainbow (1983) with the exception that in the latter study, maximal UVER factors were observed at earlier times post infection. This may be attributable to the fact that the Vag assay of the latter study was conducted at 37°C whereas this study used 33°C.

A.III. The Survival of Ad5ts36 and Ad5ts125 Viral Progeny as a Function of UV-dose to Virus in Normal Human Fibroblast Strain CRL1221

In order to study the fidelity of viral DNA repair and/or replication and the contribution of "error-prone" repair processes to the UVER responses of normal and repair deficient human fibroblasts, it was necessary to use a progeny assay measuring the phenotypic reversion of adenovirus ts mutants to wild type under experimental conditions where UVER responses were expressed. Normal human fibroblast strain CRL1221 was used to examine the kinetics of UV-dose dependent viral survival and mutagenesis among the progeny resulting from infections of UV-irradiated Ad5ts36 or Ad5t125. The normal fibroblast strain (CRL1221) was infected with unirradiated Ad5ts36 and Ad5ts125 at an average moi of 0.4. At each UV-dose to virus, a 50-fold increase in viral particles was irradiated and subsequently infected onto unirradiated CRL1221 monolayers.

Figure 2

Time courses of Ad5ts36 Vag positive cell formation for unirradiated and UV-irradiated virus in unirradiated and UV-irradiated host cell monolayers. Results for the normal human fibroblast strain Hff passage 14.

TOP PANEL   : unirradiated Ad5ts36

                ○ No UV dose to the cells

                ● 15 J/m^2 to the cells

CENTER PANEL: UV-irradiated Ad5ts36 (1.2×10^3 J/m^2)

                △ No UV dose to the cells

                ▲ 15 J/m^2 to the cells

BOTTOM PANEL: UVER factors.

Figure 2

80

Figure 2
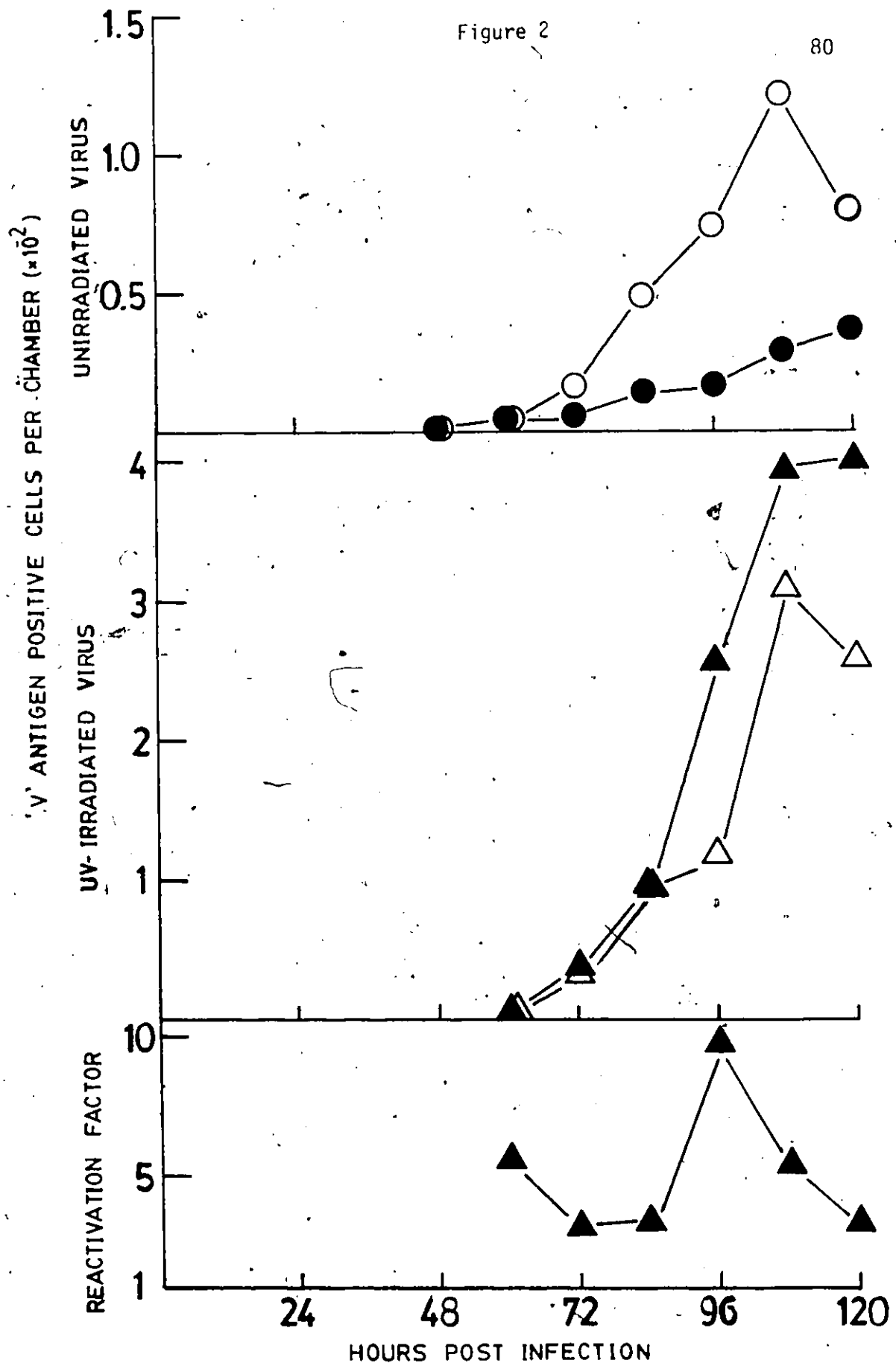
Tables 2 and 3 list the survival and UV-induced reversion among the progeny of UV-irradiated Ad5ts36 and Ad5ts125 as a function of increasing UV dose to infecting virus. UV-induced reversion frequency is defined as the reversion frequency of UV-irradiated virus minus the reversion frequency of intact virus in unirradiated cells. The progeny surviving fractions have been plotted as a function of UV-dose to the virus (Figure 3). Surviving fractions were determined by comparing the ratio of the plaque titres of UV-irradiated and unirradiated viral progeny obtained from unirradiated fibroblasts and plaqued at 33°C on HeLa cells. The UV-dose dependent plaque inactivation kinetics of Ad5ts36 and Ad5ts125 progeny from normal strain CRL1221 was consistent with single hit, exponential inactivation such that D_o's of 220 ± 20 and 270 ± 20 J/m^2 ($\pm$ 1SE) were observed for Ad5ts36 and Ad5ts125 respectively. Examination of Figure 3 and comparison of the D_o values for these two mutants suggest very similar UV survival for Ad5ts36 compared to Ad5ts125.

A.IV.   The Kinetics of UV-induced Ad5ts36 and Ad5ts125

Reversion in Normal Human Fibroblast Strain CRL1221

As well as determining progeny survival of UV-irradiated Ad5ts36 and Ad5ts125 in CRL1221, it was possible to estimate the reversion of progeny by determining the viable plaque forming ability at 39°C (nonpermissive) upon HeLa monolayers. The UV-induced reversion frequency for each UV-dose to virus was calculated as described in equation 6 (Section B) and these values can be found in Tables 2 and 3. A log-log plot of progeny UV-induced reversion frequencies versus UV fluence can be used to estimate the number of "hits" required to induce

TABLE 2

Survival and UV-induced reversion among the progeny of UV'd
Ad5ts36 produced in normal human fibroblast strain CRL1221
(passage 31).

Dose to virus (J/m^2)	Progeny (33°) Surviving Fraction	Number of Lethal Hits	UV-Induced Reversion Frequency $(x\ 10^{-5})$
0*	1.0	-	-
200	2.9×10^{-1}	1.3	3.1
400	1.4×10^{-1}	2.0	4.8
400	5.7×10^{-2}	2.9	27
600	3.1×10^{-2}	3.5	42
800	2.1×10^{-2}	3.8	84
800	3.1×10^{-2}	3.5	25
1000	1.7×10^{-2}	4.1	69
1200	3.8×10^{-3}	5.6	240
1400	6.6×10^{-4}	7.3	440
1400	2.3×10^{-3}	6.0	150
1600	2.9×10^{-4}	8.2	990

* Spontaneous Reversion Frequency = 1.2×10^{-5}

TABLE 3

Survival and UV-induced reversion among the progeny of UV'd
Ad5ts125 grown in the normal human fibroblast strain CRL1221
(passage 23).

Dose to virus (J/m^2)	Progeny (33°) Surviving Fraction	Number of Lethal Hits	UV-Induced Reversion Frequency (x 10^{-5})
0$^+$	1.0	-	0
200	1.7 x 10^{-1}	1.8	0.77
400	8.7 x 10^{-2}	2.4	1.9
600	3.9 x 10^{-2}	3.2	1.0
800	3.0 x 10^{-2}	3.5	7.9
1000	1.3 x 10^{-2}	4.3	16
1200	3.8 x 10^{-3}	5.6	41
1400	2.8 x 10^{-3}	5.9	81
1600	2.0 x 10^{-3}	6.2	N.D.*

+ Spontaneous Reversion Frequency = 6.8 x 10^{-5}
* not done

Figure 3

UV-dose dependent inactivation of Ad5ts36 and Ad5ts125 progeny production in unirradiated normal human fibroblast strain CRL1221.

The UV-inactivation of Ad5ts36 (●) and Ad5ts125 (▲) progeny production in unirradiated normal human fibroblast strain CRL1221. Progeny infectivity and subsequent plaque formation was assayed upon indicator HeLa monolayers. The average standard error associated with individual plaque determinations was ± 6.7% (Ad5ts36) or ± 8.6% (Ad5ts125) resulting in error bars contained within the data points. Linear regression analysis was used to fit survival curves to the data. Depicted is the linear regression for Ad5ts36 survival.

Figure 3
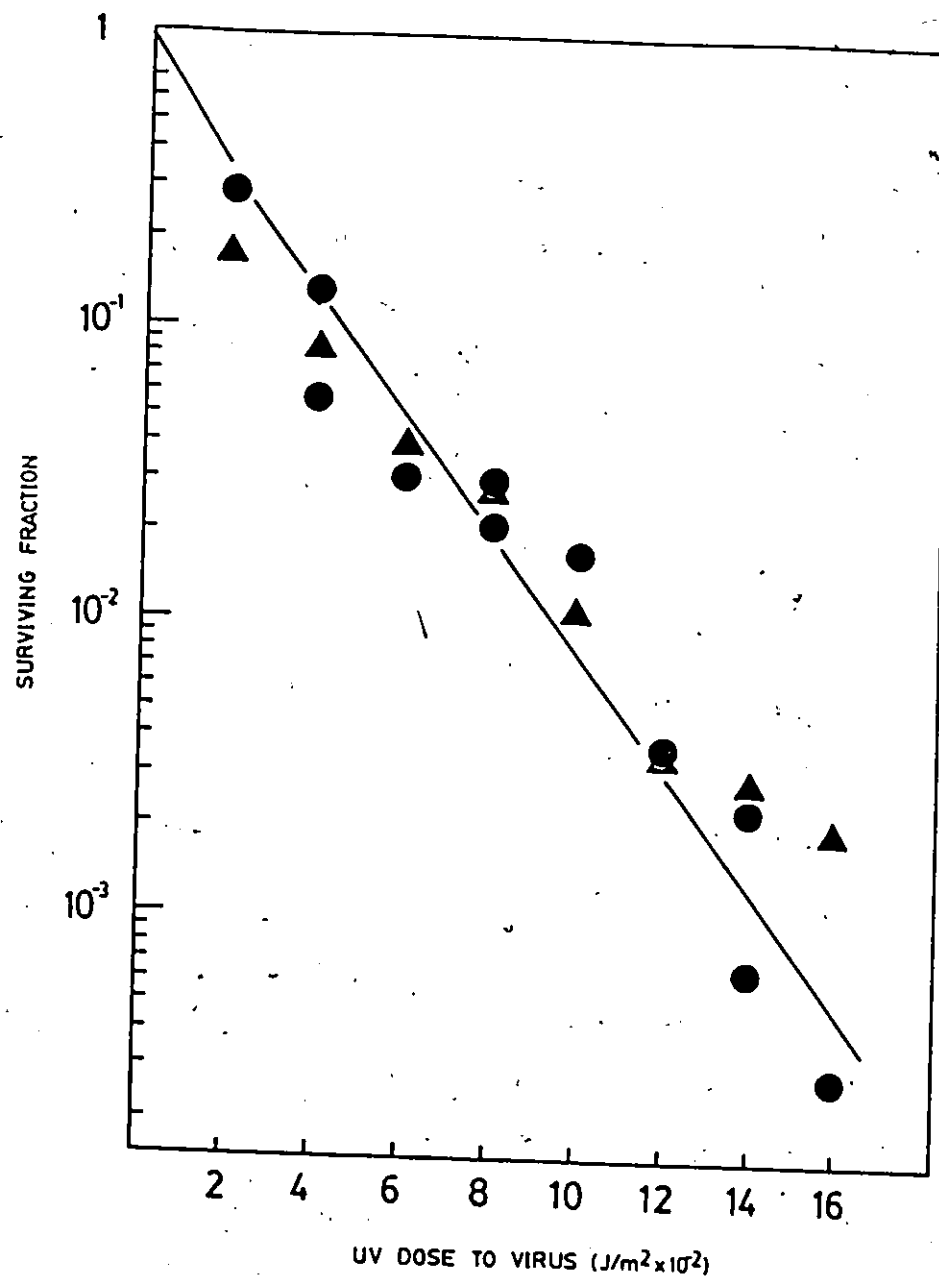
reversion among the surviving viral progeny (Cleaver and Weil, 1975; Witkin, 1976; Day and Ziolkowski, 1981; Sarasin et al, 1981; Lytle and Knott, 1982). In classical hit theory it is assumed that viral mutation is a direct consequence of the accumulation of a minimal number of physical "hits" in the virus and in the simplest "single-hit" cases, the predicted dose-response relations are exponential for survival and linear for mutation frequency (Haynes et al, 1984). The slope of the UV-induced reversion frequency versus UV-dose as plotted upon a log-log axis may be greater than one, indicating that at the level of the individual viral particle, there is a threshold for the biological response being measured (mutation). The observed dose-response curves bend smoothly away from the origin (on a linear-log plot) with zero initial slope, as the square (or some higher power) of the dose (Eckardt and Haynes, 1977; Haynes et al, 1984). A linear curve has been observed when the log of UV-induced reversion frequency was plotted versus the log UV-dose (Figure 4) of Ad5ts36 or Ad5ts125 progeny from the normal human strain CRL1221. Figure 4 indicated a dose dependent increase in UV-induced reversion frequency such that the slope of the resulting straight line was 2.5 $\pm$ 0.3 for Ad5ts36 and 2.4 $\pm$ 0.5 for Ad5ts125. This suggests that in the normal human fibroblast strain CRL1221 between 2 and 3 "hits" were required for the expression of a reversion "event" in UV-irradiated adenovirus.

Although the kinetics of log UV-induced reversion versus log UV-fluence were similar for Ad5ts36 and Ad5ts125, the Ad5ts36 produced greater reversion frequencies per unit dose (8 fold greater at 1200 J/m^2) when compared to Ad5ts125. This may suggest a larger revertable target in Ad5ts36.

Figure 4

The UV-induced reversion frequency of Ad5ts36 and Ad5ts125 progeny, after a single lytic cycle in normal human fibroblast strain CRL1221.

The UV-induced reversion frequency (RF) of Ad5ts36 (●) or Ad5ts125 (▲) progeny obtained from lytic infections of normal human fibroblast strain CRL1221 expressed as a function of UV-dose to infecting virus. The average percentage standard error for RF determinations was ± 13% (Ad5ts36) and ± 16% (Ad5ts125). Linear regression analysis produced cures with slopes of 2.5 ± 0.3 (Ad5ts36) or 2.4 ± 0.5 (Ad5ts125). Average moi for unirradiated virus in unirradiated cells was 0.4.

Figure 4
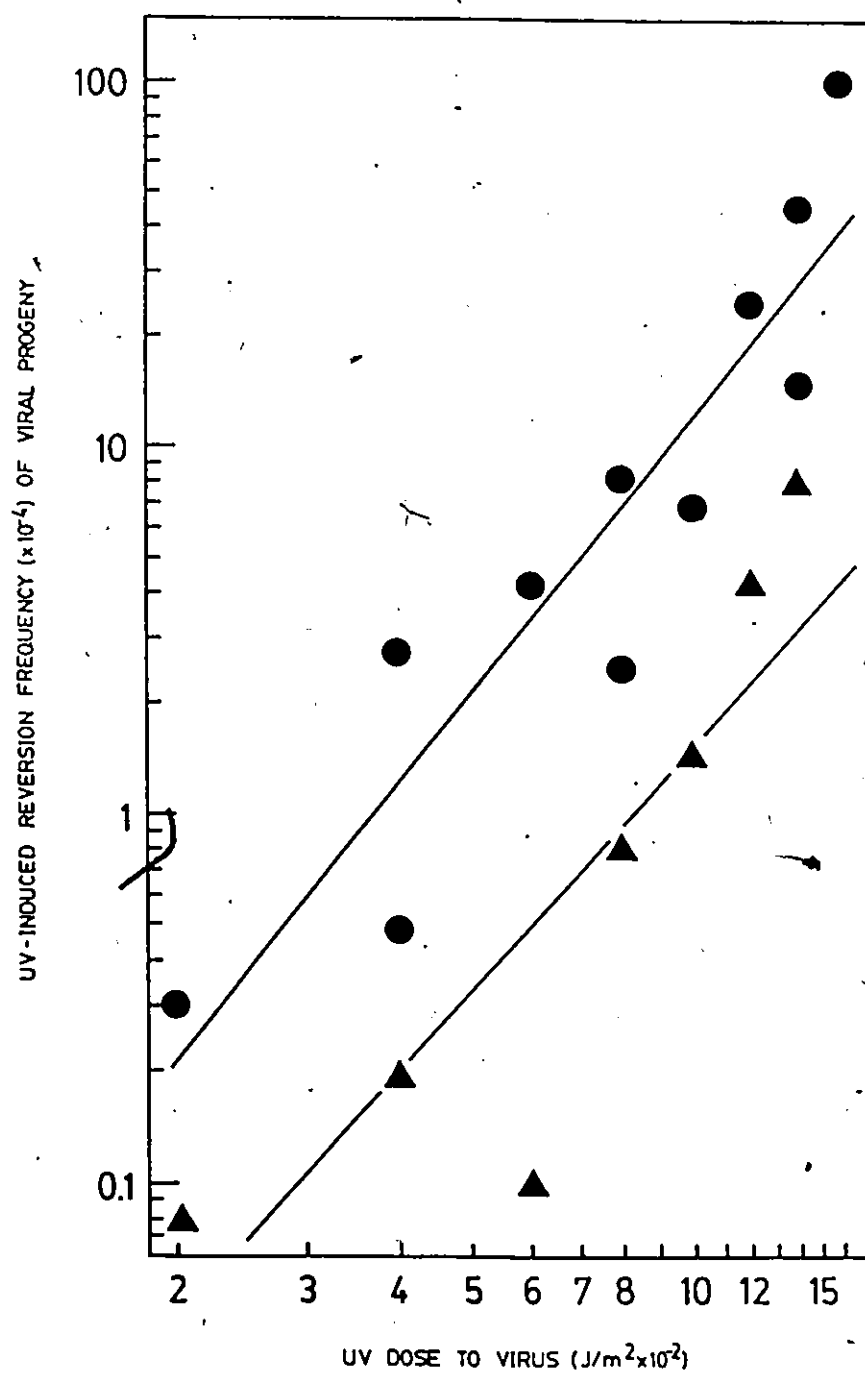
UV-INDUCED REVERSION FREQUENCY ($\times 10^4$) OF VIRAL PROGENY

UV DOSE TO VIRUS ($J/m^2 \times 10^2$)

B.I. <u>UV-enhanced Mutagenesis of UV-irradiated Adenovirus in Normal</u>

<u>Human Fibroblast Strains</u>

The UV-enhanced mutagenesis (UVEM) of Ad5ts36 in normal human fibroblast strains can be determined in a manner analogous to the determination of UVER values by determining the phenotypic reversion frequencies of unirradiated and UV-irradiated virus obtained from both UV-irradiated and control human fibroblast monolayers. The UVEM factor can be defined as:

$$UVEM = \frac{\text{Targeted Increase in Viral Reversion Frequency}}{\text{Untargeted Increase in Viral Reversion Frequency}} \qquad (1)$$

where;

$$\text{Targeted Increase} = \frac{\begin{array}{c}\text{Reversion Frequency of} \\ \text{viral progeny from} \\ \text{UV-irradiated cells infected} \\ \text{with UV-irradiated virus}\end{array}}{\begin{array}{c}\text{Reversion Frequency of} \\ \text{viral progeny from control} \\ \text{(unirradiated) cells} \\ \text{infected with UV-irradiated} \\ \text{virus}\end{array}} \qquad (2)$$

and:

$$\text{Untargeted Increase} = \frac{\begin{array}{c}\text{Reversion Frequency of} \\ \text{viral progeny from} \\ \text{UV-irradiated cells infected} \\ \text{with unirradiated virus}\end{array}}{\begin{array}{c}\text{Reversion Frequency of} \\ \text{viral progeny from control} \\ \text{(unirradiated) cells infected} \\ \text{with unirradiated-virus}\end{array}} \qquad (3)$$

The term "targeted" is used in reference to mutagenic (reversion) events associated with a UV damaged viral probe. Similarly

"untargeted" refers to mutagenic (reversion) events that are associated with a non damaged viral probe. Rearrangements of equations 1, 2 and 3 reveal that UVEM can also be defined as:

$$UVEM = \dfrac{\dfrac{\text{Reversion Frequency of viral progeny from UV-irradiated cells infected with UV-irradiated virus}}{\text{Reversion Frequency of viral progeny from UV-irradiated cells infected with unirradiated virus}}}{\dfrac{\text{Reversion Frequency of viral progeny from control cells infected with UV-irradiated virus}}{\text{Reversion Frequency of viral progeny from control cells infected with unirradiated virus}}} \qquad (4)$$

The above equation (4) is analogous to the calculation of UV-enhanced reactivation using the viral progeny titres obtained at the permissive temperature:

$$UVER = \dfrac{\text{The surviving fraction of viral progeny (33°C) from UV-irradiated cells}}{\text{The surviving fraction of viral progeny (33°C) from control (unirradiated) cells}} \qquad (5)$$

Another equation which is useful in describing the cellular response to viral mutagenic processes is:

UV-induced Reversion = Targeted Progeny Reversion (in Unirradiated

cells) - the spontaneous progeny reversion   (6)

where spontaneous (or background) reversion frequency refers to the progeny reversion observed in unirradiated cells using unirradiated virus. As a convention this thesis will use the definitions suggested by Siede et al, 1983 which states that UV-induced mutagenesis (or reversion) refers to the enhancement of mutation frequencies caused by

UV-irradiation while UV "inducibility" (or UV-inducible) refers to those proteins newly synthesized in response to UV-irradiation that are involved in the induction process.

The inactivation of viral functions (such as progeny production) by UV damage can be interpreted mathematically using hit theory and the Poisson distribution such that for the survival of UV-irradiated virus in unirradiated (control) cells:

$$\text{Surviving Fraction} \quad (N/N_o) = e^{-a} \qquad (7)$$

Where "a" is the average number of "lethal" hits per viral genome left unrepaired by cellular processes, N is the number of survivors following UV-irradiation and N_o is the total viable population before irradiation. This assumes that plaque inactivation of viral progeny is produced by a single hit in control (unirradiated) human fibroblasts and no multiplicity reactivation has occurred. From equation 7 it is apparent that the mean number of lethal hits per genome can be determined by calculating the -ln (SF) of viral progeny at any dose to the virus. Application of hit theory allows the mathematical description of dose response curves and assumes that UV photon interactions ("hits") are independent of each other and follow a Poisson distribution. The surviving fraction as calculated from the Poisson distribution is $P(o) = e^{-a}$ or the probability of an infectious particle receiving exactly zero lethal hits.

To plot a survival curve,

$$a = KD \qquad (8)$$

where K is a constant and D is the UV dose such that when In SF is plotted vs dose (D) to the virus, a straight line exponential inactivation occurs with a slope K reflecting the constitutive ability of unirradiated cells for the repair of UV-damaged virus. It should be apparent that the value "a" can be used as an alternative to UV dose to compare the survival characteristics of fibroblast cell strains which posses different constitutive DNA repair capacities.

The concept that UVER is accompanied by UVEM has been suggested for both bacteriophage (Weigle, 1953: Defais et al, 1971; Witkin, 1976) and mammalian cells (Sarasin and Benoit, 1980). The existence of an "error-prone" UVEM response concomitant with UVER would suggest the existence of a mammalian equivalent to the "SOS" repair system of bacteria which is required for the expression of phage UVER and UVEM (Witkin, 1976; Walker, 1984). Comparison of the slopes of the dose dependent increases in reversion frequencies for Ad5ts36 and Ad5ts125 (Figure 4) indicated that there was no significant difference in slope between the two mutants. Since UVEM values were determined not by the magnitude but by the ratio of targeted to untargeted increases, the mutants will express similar UVEM responses (under similar experimental conditions) if equivalent untargeted increases are observed for the two mutant viral probes. Results for normal human fibroblast strains suggest that untargeted increases were similar for both viral probes. Results were therefore grouped according to the delay in viral infection following cellular UV-induction. This allowed examination of the inducible nature of the untargeted increase, targeted increase, UVER and UVEM in normal cells.

B.II. 24 Hour Delay in Viral Infection Following UV-irradiation of
Cells

Since a 24 hour delay in viral infection allowed the expression
of UVER of UV-irradiated adenovirus in CRL1221, further normal strains
were examined under the same experimental conditions. Tables 4
contains a typical example of Ad5ts36 reversion frequencies when viral
infection of normal human fibroblast strain A2 was delayed 24 hours.
Table 4 contains the progeny titre data from control and UV-irradiated
(10 J/m^2) normal human fibroblast strain A2 infected with unirradiated
and UV-irradiated (1200 J/m^2) Ad5ts36. Standard error for the RF
determinations was by the method of Defais et al. (1971). The moi was
the same as that used for Figure 1.

The titres obtained on KB cells were used to calculate the UVER
as described in equation 5. The reversion frequencies for the
respective cell/virus treatments were used to calculate the UVEM value
as illustrated in equations 1 and 4. Values greater than 1 are
indicative of enhancement of viral mutagenesis in preirradiated cells.
The untargeted increase (if greater than 1.0) suggests a loss of
fidelity in the replication of an undamaged probe due to preirradiation
of the cells and the method of calculation is illustrated by equation
3. The induced reversion frequency is a measure of the amount of
reversion associated with UV damage to the probe in control cells
(equation 6) corrected for the background or spontaneous reversion
frequency observed in unirradiated fibroblasts for unirradiated virus.

Examination of Table 4 indicated that the reversion frequency
of unirradiated virus increased in normal fibroblasts that had received

TABLE 4

Reversion of Ad5ts36 in normal human fibroblast strain A2 (passage 22) after 96 hours of lytic growth at 33°C and titred on KB cells at the permissive and nonpermissive temperatures (24 hour delay in viral infection following UV to the cells).

UV Dose J/m^2		$39°$ pfu/ml	$33°$ pfu/ml	Reversion Frequency*
cells	virus	$(x\ 10^3)$	$(x\ 10^6)$	$(x\ 10^{-5})$
0	0	9.7	756	1.3 ± 0.04
10	0	14	590	2.4 ± 0.1
0	1200	1.4	0.950	150 ± 7
10	1200	22	6.57	340 ± 14

UVER (33°)   = 8.9 ± 1.0

UVEM   = 1.2 ± 0.2

UNTARGETED INCREASE   = 1.9 ± 0.2

TARGETED INCREASE   = 2.3 ± 0.2

UV-INDUCED REVERSION FREQUENCY = 149×10^{-5}

$SF = e^{-a}$; $a = 10.6$

$$* \pm \Delta R = R\sqrt{\left(\frac{\Delta x}{x}\right)^2 + \left(\frac{\Delta y}{y}\right)^2}$$

$$\Delta x = \frac{\sigma x}{\sqrt{n}} \qquad \Delta y = \frac{\sigma y}{\sqrt{n}}$$

(calculation from Defais et al., 1971)

x = number of plaques counted at 33°C

y = number of plaques counted at 39°C

n = number of replicate plates

a UV dose of 10 J/m^2, 24 hours before viral infection. This produced an untargeted increase of 1.9 $\pm$ 0.2 suggesting that preirradiation of normal fibroblasts caused a decrease in fidelity among the progeny resulting from infections of intact virus. UV-irradiation of virus (1200 J/m^2) in control cells caused a substantial increase, in the reversion frequency (over background), reflected by the high UV-induced reversion frequency (149 x 10^{-5}). Similarly, preirradiation of the normal fibroblasts increased the reversion frequency of UV-irradiated virus as compared to that in unirradiated cells. The magnitude of the targeted increase (2.3 $\pm$ 0.2) was not significantly greater than the untargeted increase and therefore resulted in a UVEM not significantly greater than 1 (1.2 $\pm$ 0.2). This suggested that UV-irradiation of a single normal human fibroblast strain (A2) did not increase the mutagenesis of progeny from a UV damaged probe as compared to an undamaged probe by a factor greater than about 1.4. The EM effect, if it existed, was small and could not be determined with any statistical significance in a single experiment due to the errors involved. It was striking that a large UVER value was found (8.9) accompanying a UVEM of 1.2 indicating that a high UVEM value does not necessarily accompany a high UVER.

Table 5 lists the parameters of adenovirus UV mutagenesis (reversion) using 5 normal human fibroblasts strains obtained in 10 separate experiments where viral infections were delayed 24 hours following cellular UV-irradiation. These parameters include untargeted increase, targeted increase, UVER and UVEM.

It can be seen that pooled data for normal fibroblast strains (Table 5) infected 24 hours following UV-irradiation produced an mean

TABLE 5

PARAMETERS OF ADENOVIRAL UV-MUTAGENESIS IN NORMAL HUMAN FIBROBLASTS

CELLS	UV DELAY (HRS)	UV DOSE (J/m^2) CELLS	VIRUS	UNTARGETED INCREASE	TARGETED INCREASE	UVER	UVEM
A2	24	10	1200	1.9	2.3	8.9	1.2
A2	24	10	1200	1.4	1.1	1.9	0.76
CRL1221	24	10	1200	2.7	2.6	0.67	0.96
CRL1221	24	10	600	2.8	4.2	4.0	1.5
GM2674	24	10	1200	1.2	2.4	1.5	2.1
GM2674	24	10	1200	1.0	1.1	3.5	1.1
GM969	24	10	1200	1.9	1.7	5.8	0.95
GM969	24	10	600	0.60	1.1	6.0	1.8
GM288	24	10	1200	1.7	1.4	1.1	0.82
CRL1221*	24	10	1200	0.34	0.79	0.48	2.3
POOLED MEANS ± 1 SE				1.6 ± 0.3	1.9 ± 0.3	3.4 ± 0.8	1.4 ± 0.2

* AD5TS125 INFECTION

untargeted increase of 1.6 ± 0.3, a targeted increase of 1.9 ± 0.3, a UVER of 3.4 ± 0.8 and a UVEM of 1.4 + 0.2 (+ 1SE). A UVER significantly greater than 1 was observed concomitantly with a UVEM which was also greater than one (1.4 ± 0.2). This pooled data indicates a small but significant UVEM in normal human fibroblasts that were UV-irradiated 24 hours prior to adenovirus infection.

B.III. Immediate Viral Infection Following UV to the Cells

Table 6 contains data that was typically observed after immediate infection of normal fibroblast strain CRL1221 using the adenovirus progeny assay. Strain CRL1221 was UV-irradiated (10 J/m^2) or left unirradiated and immediately infected with intact or UV-irradiated (1200 J/m^2) Ad5ts36. The CRL1221 strain expressed an untargeted increase of (3.9 ± 0.2) and a targeted increase of (0.85 ± 0.1) when viral infections were immediately following UV-irradiation of the cells. In this assay the magnitude of UVER and UVEM were both significantly less than one, indicating that UV-irradiation of the cells inhibited the expression of UVER and UVEM after immediate infections. The reduced UVEM found in Table 6 was significantly smaller than the UVEM values found for pooled normals when viral infection was delayed 24 hours after UV to the fibroblast cells (Table 5).

Progeny data for 5 separate experiments obtained from immediate infections of Ad5ts36 or Ad5ts125 in 2 normal cell strains were pooled (Table 7). The pooled means (± 1SE) of the various parameters of adenoviral mutagenesis were 2.0 ± 0.5 for the untargeted increase, 0.93 ± 0.13 for the targeted increase, 1.2 ± 0.4 for the UVER and 0.61 ±

TABLE   6

Reversion of Ad5ts36 in normal human fibroblast strain
CRL1221 (passage 31) after 96 hours of lytic growth at 33°C
and titred on HeLa cells at the permissive and nonpermissive
temperatures (immediate viral infection following UV to the
cells).

| UV Dose J/m^2 | | 39° pfu/ml | 33° pfu/ml | Reversion Frequency* |
cells	virus	(x 10^3)	(x 10^7)	(x 10^{-4})
0	0	5.86	3.4	1.7 ± 0.02
10	0	42	6.21	6.7 ± 0.1
0	1200	16	1.3	13 ± 0.4
10	1200	18	1.6	11 ± 0.7

UVER (33°)   = 0.68 ± 0.04

UVEM   = 0.22 ± 0.02

UNTARGETED INCREASE   = 3.9 ± 0.2

TARGETED INCREASE   = 0.85 ± 0.08

UV-INDUCED REVERSION FREQUENCY = 109 x 10^{-5}

LETHAL HITS (a) = 4.9

* ± ΔR

0.16 for the UVEM. Comparison of Tables 5 and 7 indicates that targeted increase, UVER and UVEM showed a significant increase for delayed as compared to immediate infections while the untargeted increase was not significantly altered. This suggests that the untargeted and targeted responses may arise, in part from separate mechanisms.

B.IV. Time Course for Adenovirus UVER and UVEM in Normal Fibroblast Strains

Since UVEM and UVER significantly decreased upon immediate as compared to 24 hour delayed viral infection, we examined other infection delay periods (12,36,48 hours) to determine the optimal time for maximal expression of UVER/UVEM responses.

Table 8 contains typical progeny data for normal fibroblast strain CRL1221 infected 12 hours following cellular UV-irradiation with intact or UV-irradiated adenovirus. The mean values of the untargeted increase, targeted increase, UVER and UVEM can be found in Table 7 for this infection delay.

Reversion frequencies were also obtained from CRL1221 infected with UV-irradiated or intact Ad5ts125, 36 hours (Table 9) or 48 hours after cellular UV-irradiation (Table 10). The parameters of adenovirus mutagenesis (UVER, UVEM, untargeted increase, targeted increase) have been plotted as a function of the time delay between cellular UV-irradiation and viral infection by combining the data of sections B.I. to B.IV. (Figure 5). It can be seen from Figure 5 that the maximal UVER and UVEM responses were found after a UV delay of 24 hours. Examination of Figure 5 suggests that the time course of

TABLE 7

PARAMETERS OF ADENOVIRAL UV-MUTAGENESIS IN NORMAL HUMAN FIBROBLASTS

CELLS	UV DELAY (HRS)	UV DOSE (J/M^2) CELLS	VIRUS	UNTARGETED INCREASE	TARGETED INCREASE	UVER	UVEM
GM288	0	10	1200	1.0	1.2	0.67	1.2
CRL1221	0	10	1200	3.9	0.85	0.68	0.22
CRL1221*	0	10	1200	2.6	1.0	0.91	0.38
CRL1221*	0	10	1200	0.92	0.40	0.69	0.44
CRL1221*	0	10	1200	1.5	1.2	3.0	0.82
POOLED MEANS ± 1 SE				2.0 ± 0.5	0.93 ± 0.13	1.2 ± 0.4	0.61 ± 0.16
CRL1221*	12	10	1200	3.2	2.5	0.88	0.80
GM288	12	10	1200	0.84	0.68	1.1	0.80
POOLED MEANS ± 1 SE				2.0 ± 0.8	1.6 ± 0.6	0.99 ± 0.08	0.80

* AD5TS125 INFECTIONS

TABLE 8

Reversion of Ad5ts125 progeny from the normal human
fibroblast strain CRL1221 (passage 25) after 96 hours of
lytic growth at 33°C and titred on HeLa cells at the
permissive and nonpermissive temperatures (12 hour delay
in viral infection following UV to the cells).

| UV Dose J/m^2 | | $39°$ pfu/ml | $33°$ pfu/ml | Reversion Frequency* |
cells	virus	$(x\ 10^3$	$(x\ 10^6)$	$(x\ 10^{-4})$
0	0	6.13	19	3.2 ± 0.1
10	0	11.1	10.8	10.3 ± 0.3
0	1200	3.4	6.48	5.2 ± 0.2
10	1200	4.3	3.3	13 ± 0.3

UVER (33°)	=	0.88 ± 0.09
UVEM	=	0.80 ± 0.11
UNTARGETED INCREASE	=	3.2 ± 0.2
TARGETED INCREASE	=	2.5 ± 0.2
UV-INDUCED REVERSION FREQUENCY	=	19×10^{-5}

LETHAL HITS (a) = 5.0

* $\pm \Delta R$

TABLE 9

Reversion of Ad5ts125 progeny from the normal human fibroblast strain CRL1221 (passage 25) after 96 hours of lytic growth at 33°C and titred on HeLa cells at the permissive and nonpermissive temperatures (36 hour delay in viral infection following UV to the cells).

| UV Dose J/m^2 | | 39° pfu/ml | 33° pful/ml | Reversion |
cells	virus	(x 10^3)	(x 10^6)	Frequency* (x 10^{-4})
0	0	4.6	25	1.8 ± 0.03
10	0	4.6	28	1.6 ± 0.02
0	1200	4.1	9.00	4.6 ± 0.1
10	1200	4.0	8.15	4.9 ± 0.1

UVER (33°) = 0.82 ± 0.04

UVEM = 1.2 ± 0.1

UNTARGETED INCREASE = 0.90 ± 0.04

TARGETED INCREASE = 1.1 ± 0.04

UV-INDUCED REVERSION FREQUENCY = 28 x 10^{-5}

LETHAL HITS (a) = 4.9

* ± ΔR

TABLE 10

Reversion of Ad5ts125 progeny from the normal human
fibroblast strain CRL1221 (passage 25) after 96 hours of
lytic growth at 33°C and titred on HeLa cells at the
permissive and nonpermissive temperatures (48 hour delay
in viral infection following UV to the cells).

UV Dose J/m^2		39° pfu/ml (x 10^3)	33° pfu/ml (x 10^6)	Reversion Frequency* (x 10^{-5})
cells	virus			
0	0	6.48	34	1.9 ± 0.1
10	0	3.5	15	2.3 ± 0.07
0	1200	7.42	12.9	5.75 ± 0.16
10	1200	5.29	8.58	6.17 ± 0.17

UVER (33°)   = 1.5 ± 0.2

UVEM   = 0.88 ± 0.14

UNTARGETED INCREASE   = 1.2 ± 0.1

TARGETED INCREASE   = 1.07 ± 0.06

UV-INDUCED REVERSION FREQUENCY   = 38 x 10^{-5}

LETHAL HITS (a) = 4.9

* ± ΔR

Figure 5

The time course expression of parameters of UV mutagenesis in pooled normal human fibroblasts using Ad5ts36 and Ad5ts125 as molecular probes.

UPPER PANEL: Progeny UVEM (●) and UVER (○) factors plotted as a function of the delay of infection following UV to the fibroblasts.

LOWER PANEL: Progeny targeted (▲) and untargeted (△) increases plotted as a function of the delay of infection following UV to the fibroblasts.

Figure 5   102
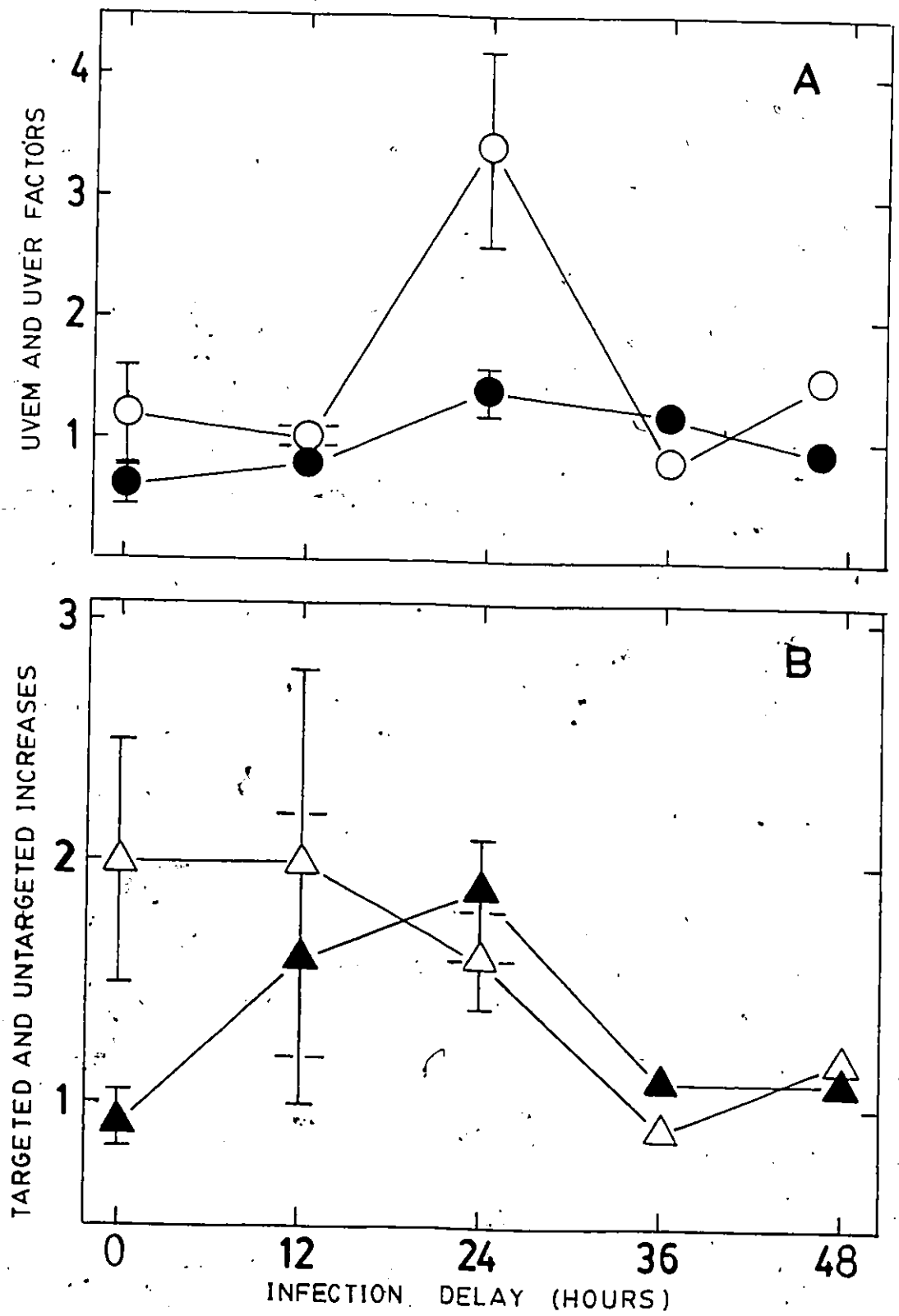
UVER/UVEM responses parallels most closely the time course for targeted increase. Maximal untargeted and targeted increases were observed using immediate and 24 hour delayed viral infection protocols, respectively. The maximal targeted increase was concomitant with maximal UVER and UVEM responses. The difference observed in the time course of untargeted and targeted increases following cellular irradiation of normal fibroblast cells suggests that separate mechanisms, at least in part were responsible for these parameters of adenovirus mutagensis. Furthermore, these results suggested that examination of repair deficient cell strains for UVER and UVEM responses should be under experimental conditions (24 hour delayed viral infection) that maximized these responses in normal cells.

B.V. Culture Conditions Affecting Reversion Frequencies of Intact and UV-irradiated Virus

Variation in the spontaneous (background) RF has been previously observed using an adenovirus progeny assay (Day and Ziolkowski, 1981). Similar variation (1.3×10^{-5} to 4.2×10^{-3}) in the spontaneous RF was observed in this study for viral progeny obtained from the infection of undamaged virus in normal human fibroblasts using the same viral stock. In order to examine the effects of culture conditions on the background reversion frequencies, control and UV-irradiated normal cells were infected with intact Ad5ts36 such that the cells were plated for increasing time periods. Table 11 lists the time course of untargeted increases as a function of the length of cellular plating times in hours.

Normal human fibroblast strain CRL1221 was grown in 60 mm plates

until confluency. At 24 hour intervals paired monolayers were either UV-irradiated (10 J/m^2) or left unirradiated and fresh complete alpha-MEM was added to each plate. Intact virus was infected 24 hours after UV to the cells. Progeny from each viral infection were collected at 96 hours p.i. and assayed at 33°C or 39°C using HeLa indicator cells. The reversion frequencies of intact virus in unirradiated and UV-irradiated cells (24 hour delayed infection) can be found in Table 11 expressed as a function of increased cellular plating times (hours). Increasing length of cellular plating time increased the spontaneous progeny reversion frequencies in both unirradiated and UV-irradiated cells. As plating time increased from 48 to 144 hours, the RF in unirradiated cells also increased from 8.7 to 459 x 10^{-5}. Similarly, the RF in UV-irradiated cells increased from 9.0 to 770 x 10^{-5} during the same time period. Although RF increased in both unirradiated and UV-irradiated cells, untargeted response was similar during the time course (Table 11).

During the normal fibroblast time course (Figure 5), the RF of UV-irradiated virus (1200 J/m^2) in unirradiated cells was very constant for the 12-48 hour time points (the average RF in unirradiated CRL1221 for UV-irradiated virus was [31 ± 5] x 10^{-5} ± 1SE). When UV-irradiated virus infected unirradiated CRL1221 using the immediate infection protocol, the RF of UV-irradiated virus was 10 fold higher (383 x 10^{-5}). This increase in mutagenesis may be related to the length of cellular plating time without the addition of fresh alpha-MEM since immediate infections were refed only at the time of cellular irradiation. Cells from other time points were reincubated at (and refed) 37°C for 12, 24 or 36 hours before infection. Similar results

TABLE 11

The untargeted increase in reversion of Ad5ts36 progeny from UV-irradiated CRL1221 (passage 20) expressed as a function of increased cellular plating times (viral infection delayed 24 hours post UV to cells).

Plating Delay (Hours) Before Infection	UV Dose to cells (J/m^2)	39° pfu/ml* $(\times 10^3)$	33° pfu/ml* $(\times 10^6)$	Reversion Frequency $(\times 10^{-4})$	Untargeted Increase
48	0	4.6 ± 0.8	5.2 ± 0.2	8.7	-
	10	5.5 ± 0.2	4.4 ± 0.2	9.0	1.0
72	0	25 ± 3	6.1 ± 0.05	40	-
	10	52 ± 5	6.3 ± 0.6	82.9	2.0
96	0	260 ± 15	31 ± 5	82	-
	10	280 ± 21	21 ± 1	140	1.6
120	0	542 ± 57	25 ± 1	220	-
	10	700 ± 30	18 ± 1	400	1.8
144	0	483 ± 44	10.5 ± 1	459	-
	10	440 ± 22	5.75 ± 0.31	770	1.7

* ± 1SE

were obtained using normal strain GM288 where the RF for UV-irradiated virus in unirradiated cells were 73×10^{-5} (immediate), 59×10^{-5} (12 hour delay) and 39×10^{-5} (24 hour delay). These results suggest that the culture conditions may influence the RF associated with unirradiated and UV-irradiated normal human fibroblasts.

B.VI. UV-enhanced Mutagenesis of UV-irradiated Adenovirus in Cockayne's Syndrome Fibroblast Strain GM2838

After the examination of UVER and UVEM responses associated with normal human fibroblasts, it was of interest to use the same assay to probe the replication and/or repair of UV-irradiated adenovirus in fibroblasts isolated from patients with autosomal recessive DNA-repair disorders. Cockayne's Syndrome (CS) fibroblasts (GM2838) were selected since elevated levels of UV-induced cellular mutagenesis has been identified in these fibroblasts (Arlett, 1980). CS cells also display altered expression of UVER in that the maximum of the UVER dose response was shifted to UV doses (to the host cells) which were lower than the UV dose corresponding to the maximum of the profile for normal fibroblasts (Jeeves and Rainbow, 1983b). CS cells also display abnormal HCR of UV-irradiated adenovirus (Day et al, 1981; Rainbow and Howes, 1982). The same progeny technique as used for the previous normal strains was employed for CS strain GM2838.

Typical RF results from a representative experiment using Ad5ts36 as a probe of CS fibroblasts can be found in Table 12. The results of three separate Ad5ts36 progeny experiments in CS fibroblasts (GM2838) have been summarized in Table 13. Surviving fractions of viral progeny resulting from infections of UV-irradiated Ad5ts36 in

unirradiated CS monolayers have been compared to that obtained in unirradiated pooled normals (Figure 6). In 2 CS experiments the UV-dose to virus was 400 J/m^2, while in one experiment the probe was irradiated to a dose of 1200 J/m^2, similar to the dose used in the majority of normal experiments (Table 5). At both viral UV doses, the survival of progeny was reduced in CS as compared to pooled normals (Figure 6). This agrees with the data of Day et al. (1981) and Rainbow and Howes, (1982) describing reduced HCR of UV-irradiated adenovirus in CS fibroblasts. The UV-induced reversion of Ad5ts36 in unirradiated CS cells was similar to that found in normal strains when the UV-induced RF is plotted either as a function of UV-dose (Figure 7) or number of lethal hits (Figure 8).

Examination of the parameters of UV-mutagenesis in CS fibroblast strain GM2838 (Tables 12, 13) suggest that under the conditions of these assays, untargeted increase, targeted increase, UVER and UVEM appeared similar to normals that were also infected 24 hours following cellular UV-irradiation. A dose dependent increase in cellular UV-irradiation of CS fibroblasts (from 5 to 10 J/m^2) resulted in an increase in both untargeted and targeted responses but not in UVER or UVEM. Since CS fibroblasts appeared normal with respect to induced mutagenesis of adenovirus, other repair deficient human fibrolasts were examined for abnormal mutagenesis.

B.VII. UV-enhanced Mutagenesis of UV-Irradiated Adenovirus in Ataxia telangiectasia Fibroblasts

Ataxia telangiectasia (AT) fibroblasts strains have been found to be hypersensitive and hypomutable following gamma-irradiation

TABLE 12

Reversion of Ad5ts36 in Cockayne's syndrome fibroblast strain GM2838 (passage 14) after 96 hours of lytic growth at 33°C and titred on HeLa cells at the permissive and nonpermissive temperatures (24 hour delay in viral infection following UV to the cells).

| UV Dose J/m^2 | | 39° pfu/ml | 33° pfu/ml | Reversion Frequency* |
cells	virus	(x 10^2)	(x 10^7)	(x 10^{-5})
0	0	9.6	8.69	1.1 ± 0.04
5	0	5.6	4.8	1.2 ± 0.05
0	400	48.5	6.02	8.05 ± 0.23
5	400	64.8	6.08	10.6 ± 0.1

UVER (33°)   = 1.8 ± 0.1

UVEM   = 1.2 ± 0.1

UNTARGETED INCREASE   = 1.1 ± 0.1

TARGETED INCREASE   = 1.3 ± 0.5

UV-INDUCED REVERSION FREQUENCY = 6.9 x 10^{-5}

LETHAL HITS (a) = 4.3

* ± ΔR

Figure 6

Surviving fractions of viral progeny obtained from infections of unirradiated normal, AT, Cockayne's and XP fibroblasts with UV-irradiated Ad5ts36.

◯ Pooled normal strains

  (CRL1221, A2, GM2674, GM969, GM288)

● Cockayne's Syndrome (GM2838)

⬢ XP (group A) (XP25RO)

▲ Ataxia telangiectasia (AT3BI)

◆ XP (variant) (CRL1162)

Mean Surviving Fractions are shown ± 1SE

Figure 6
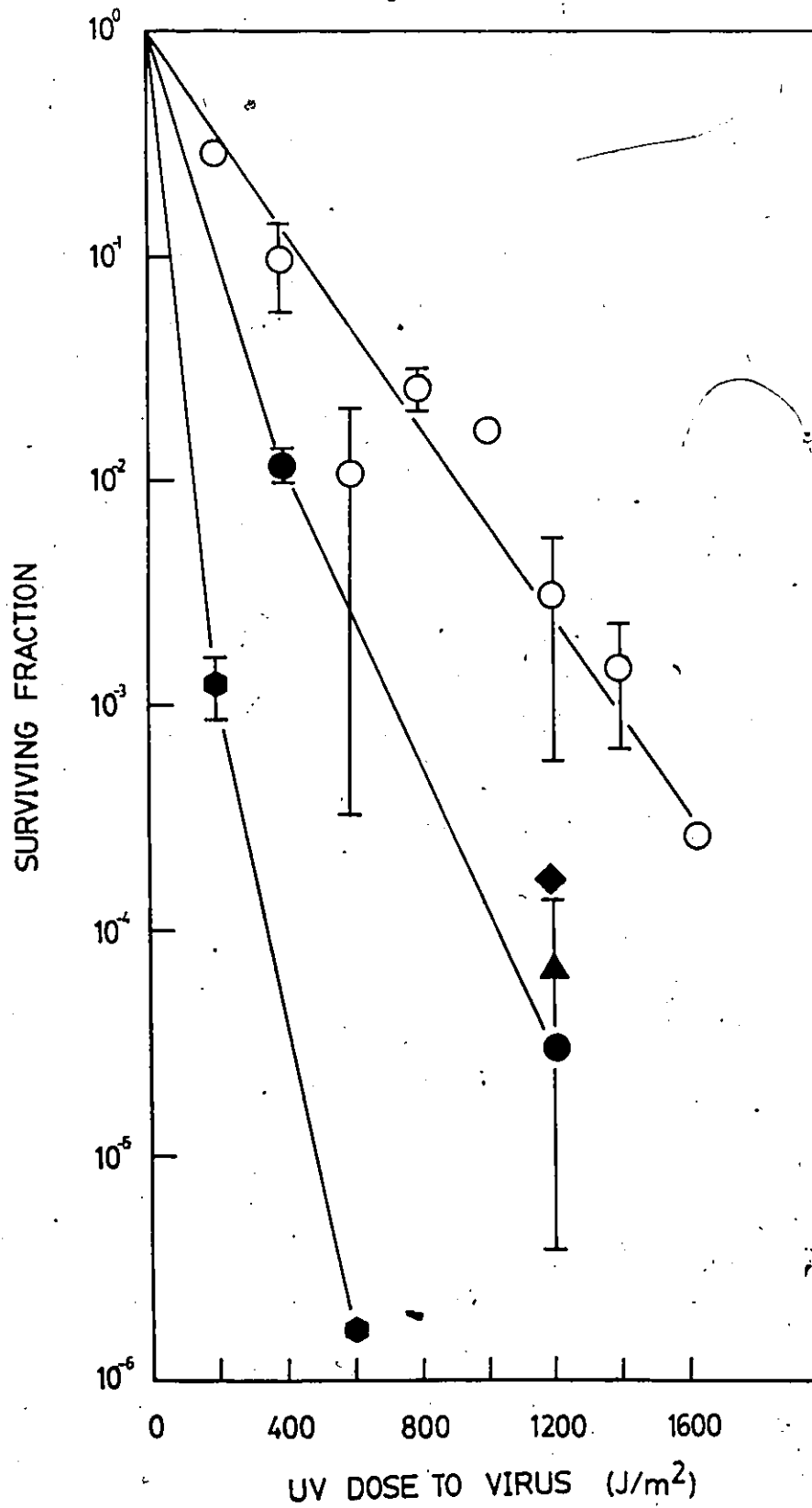
Figure 7

The UV-induced reversion frequency of Ad5ts36 progeny plotted as a function of UV dose to the virus in various normal (open symbols) and repair deficient (closed symbols) human fibroblasts.

○ CRL1221 (Normal)

⬡ GM969 (Normal)

▽ GM2674 (Normal)

◇ A2 (Normal

□ GM288 (Normal)

● GM2838 (Cockayne's Syndrome)

◆ CRL1162 (XP variant)

⬢ XP25RO (XP group A)

▲ AT3BI (Ataxia telangiectasia)

Figure 7
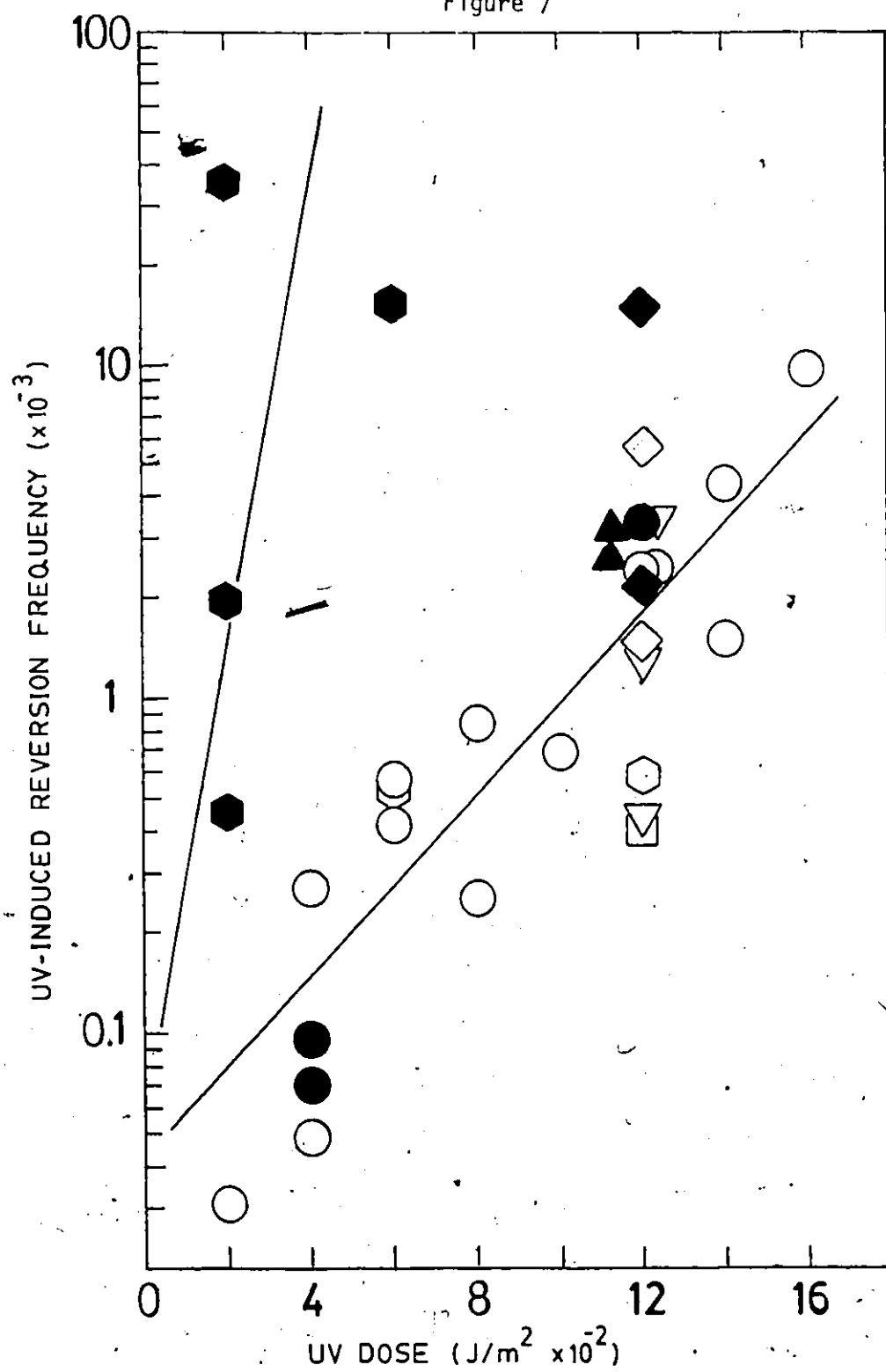
Figure 8

Ad5ts36 UV-induced reversion frequency plotted as a function of progeny "Lethal hits" (a); Open symbols are normal human fibroblast strains. Closed symbols are DNA-repair deficient human fibroblast strains.

◇ A2   (Normal)

▽ GM2674   (Normal)

○ CRL1221   (Normal)

⬡ GM969   (Normal)

☐ GM288   (Normal)

● GM2838   (Cockayne's Syndrome)

⬣ GM710   (XP group A)

◆ CRL1162   (XP Variant)

▲ AT381   (Ataxia telangiectasia)
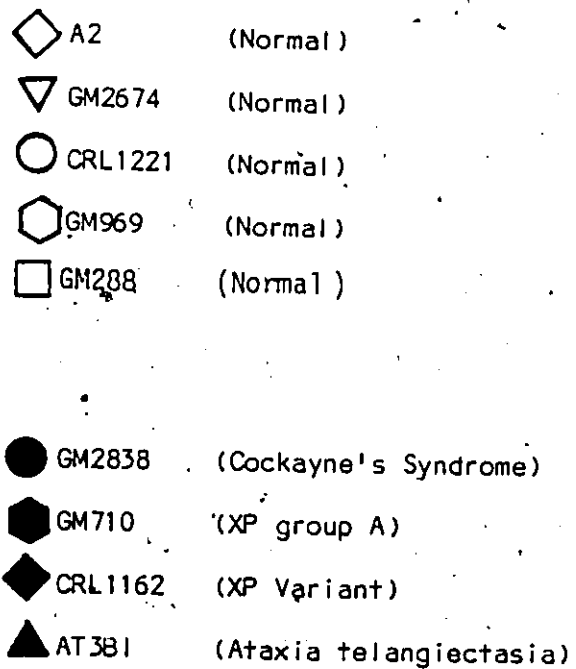
Figure 8
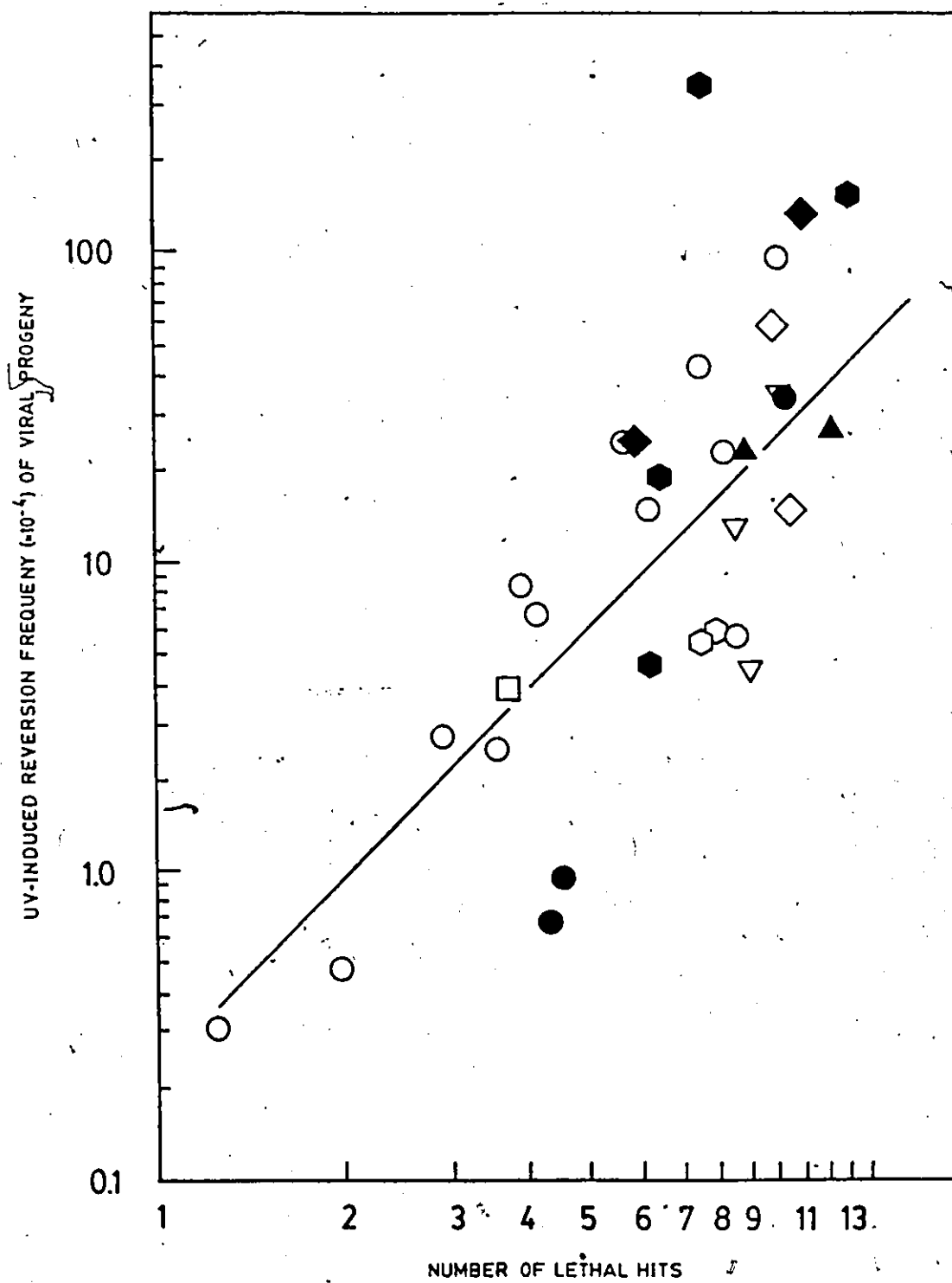
TABLE 13

PARAMETERS OF ADENOVIRAL UV-MUTAGENESIS IN VARIOUS REPAIR DEFICIENT HUMAN FIBROBLAST STRAINS

(VIRAL INFECTIONS DELAYED 24 HOURS AFTER CELLULAR UV-IRRADIATION)

CELLS	UV DELAY (HOURS)	UV DOSE (J/m^2) CELLS	VIRUS	UNTARGETED INCREASE	TARGETED INCREASE	UVER	UVEM
POOLED NORMALS							
Means ± 1SE	24			1.6 ± 0.3	1.9 ± 0.3	3.4 ± 0.8	1.4 ± 0.2
COCKAYNE'S SYNDROME							
GM2838	24	5	400	1.1	1.3	1.8	1.2
GM2838	24	5	400	1.5	1.7	1.4	1.0
GM2838	24	10	1200	2.7	2.7	1.7	1.1
ATAXIA TELANGIECTASIA							
AT3BI	24	10	1200	1.6	0.44	4.2	0.26
AT3BI	24	10	1200	1.5	0.25	1.87	0.17
AT3BI*	24	10	800	2.1	1.1	1.4	0.51
XERODERMA PIGMENTOSUM (Variant)							
CRL1162	24	10	1200	1.3	0.95	1.8	0.72
CRL1162	24	10	1200	2.0	0.65	2.3	0.33
XERODERMA PIGMENTOSUM (Complimentation Group A)							
GM710	24	0.4	200	1.3	1.1	2.2	0.83
GM710	24	0.4	200	2.6	1.6	0.68	0.62
GM710	24	0.2	600	1.4	1.2	0.98	0.80

* Ad5ts125

TABLE 14

Reversion of Ad5ts36 in ataxia telangiectasia fibroblast
strain AT3BI (passage 12) after 96 hours of lytic growth
at 33°C and titred on HeLa cells at the permissive and
nonpermissive temperatures (24 hour delay in viral infection
following UV to the cells).

UV Dose J/m^2		39° pfu/ml	33° pfu/ml	Reversion Frequency*
cells	virus	$(\times 10^2)$	$(\times 10^5)$	$(\times 10^{-6})$
0	0	4.8	2700	1.8 ± 0.04
10	0	9.4	3200	2.9 ± 0.07
0	1200	1.5	0.558	2700 ± 40
10	1200	3.2	2.8	1200 ± 50

UVER (33°)   = 4.2 ± 0.3

UVEM   = 0.26 ± 0.07

UNTARGETED INCREASE   = 1.6 ± 0.3

TARGETED INCREASE   = 0.44 ± 0.03

UV-INDUCED REVERSION FREQUENCY = 269×10^{-5}

LETHAL HITS (a) = 12.4

* $\pm \Delta R$

(Arlett, 1980). Similarly AT strains have demonstrated an aberration in gamma-ray ER of UV or gamma-irradiated Ad2 (Rainbow et al. 1983). Furthermore, HCR of UV-irradiated Ad2 is reduced in some AT strains when compared to normals (Rainbow, 1981). These DNA repair abnormalities suggested that it would be of interest to examine AT fibroblasts for UVER and UVEM of UV-irradiated adenovirus in a manner similar to that used for normal strains (Table 5).

The AT fibroblast strain AT3BI was UV-irradiated (at confluency) with 10 J/m^2, refed and infected 24 hours later with both intact and UV-irradiated (1200 J/m^2) virus. Typical results for a single experiment using Ad5ts36 as a probe can be found in Table 14. Pooled results for three separate AT3BI experiments listing the parameters of UV-induced mutagenesis (untargeted increase, targeted increase, UVER and UVEM) can be found in Table 13.

UV-irradiation of Ad5ts36 at a dose of 1200 J/m^2 resulted in a progeny survival in AT3BI that was less than that observed for pooled normals (Figure 6). UV-induced RF's of Ad5ts36 in unirradiated AT3BI were similar to normals when compared either on a per unit dose basis (Figure 7) or as a function of progeny survival (Figure 8).

Examination of the RF data obtained for a single experiment in AT3BI (Table 14) indicated that UV-irradiation of monolayers 24 hours prior to infection induced the expression of an untargeted increase (1.6 ± 0.3) but was markedly deficient in expression of a targeted increase (0.44 ± 0.03). UV-irradiation of AT increased the mutagenesis associated with an intact viral probe but reduced the mutagenesis associated with a UV damaged probe which is reflected by a UVEM value significantly less than one (0.26 ± 0.07, Table 14). This reduced UVEM

(as compared to normals) occurred even in the presence of UVER
(4.2 ± 0.3, Table 14). Similarly, pooling of three experiments in
AT3BI (Table 13) where viral infections were delayed 24 hours after
cellular irradiation resulted in an average untargeted increase of
1.7 ± 0.6 (±1SE) and an average targeted increase of 0.60 ± 0.43 (Table
13). This resulted in a UVEM that was significantly reduced
(0.31 ± 0.1) in pooled AT3BI as compared to pooled normals (1.4 ± 0.2)
under similar experimental conditions. UVER (2.5 ± 0.9) in pooled
AT3BI was concomitant with reduced UVEM and suggests that these
processes may occur through separate mechanisms in this repair
deficient strain.

The hypomutability of progeny obtained after infections of
UV-irradiated virus in UV-irradiated (but not unirradiated) AT3BI
(Figures 7 and 8) suggests that these fibroblasts may lack a
UV-inducible error-prone repair system. Alternatively, AT3BI may
posses a UV-inducible error-free repair mechanism that is specific for
UV-damaged viral templates. In either case, this inducible process
does not interfere with the expression of UVER.

B.VIII. UV Enhanced Mutagenesis of UV-irradiated Adenovirus in
XP (variant) Strain CRL1162

Xeroderma pigmentosum variant strains have been found to be
UV-hypermutable as compared to normals and it has been suggested that
the defect in XP variants may allow the expression of an error-prone
repair process (Maher et al., 1976) XP variant fibroblast strain
CRL1162 was therefore examined for UVEM of UV-irradiated adenovirus in
a manner similar to that previously used for normals.

Table 15 contains typical progeny RF values obtained for infections of intact or UV-irradiated virus in unirradiated or UV-irradiated (10 J/m^2) CRL1162 monolayers. Viral infections were delayed 24 hours following cellular UV-irradiation and the results of two separate experiments have been summarized in Table 13.

A UV dose of 1200 J/m^2 to the virus resulted in a lower (but not significantly lower) progeny survival in unirradiated CRL1162 when compared to that obtained in normals (Figure 6). The UV-induced RF was similar to that observed for normals whether plotted as a function of UV-dose to the infecting virus (Figure 7) or progeny survival (Figure 8). These results indicated that UV-induced reversion of Ad5ts36 in unirradiated XP variant strain CRL1162 was not hypermutable.

The means ($\pm$ 1SE) of the various parameters of adenoviral mutagenesis were calculated for the Ad5ts36 infections delayed 24 hours following UV-irradiation of the CRL1162 monolayers (Table 13). The mean untargeted increase for the XP variant strain was 1.6 $\pm$ 0.4 which was similar to that observed for pooled normals (1.6 $\pm$ 0.3, Table 5). The pooled targeted increase of XP variants (0.80 $\pm$ 0.2) was significantly less than the targeted increase observed for pooled normals (1.9 $\pm$ 0.3, Table 5). This produced a UVEM (0.52 $\pm$ 0.20) that was significantly less than that observed for pooled normals (1.4 $\pm$ 0.2) under similar experimental conditions. The UVER of XP variants (2.1 $\pm$ 0.3) was also smaller than that observed for normals (3.4 $\pm$ 0.8), but not significantly smaller.

XP variant fibroblasts were thus capable of expressing increased reversion associated with an undamaged viral probe in monolayers UV-irradiated 24 hours prior to infection but were unable to

TABLE 15

Reversion of Ad5ts36 in xeroderma pigmentosum strain
CRL1162 (variant, passage 16) after 96 hours of lytic
growth at 33°C and titred on HeLa cells at the permissive
and nonpermissive temperatures (24 hour delay in viral
infection following UV to the cells).

| UV Dose J/m^2 | | $39°$ pfu/ml | $33°$ pfu/ml | Reversion Frequency* |
cells	virus	$(x\ 10^3)$	$(x\ 10^5)$	$(x\ 10^{-3})$
0	0	693	896	7.74 ± 0.1
10	0	926	600	15.4 ± 0.2
0	1200	1.4	0.64	23 ± 0.9
10	1200	1.5	0.99	15 ± 0.6

UVER (33°)   = 2.3 ± 0.2

UVEM   = 0.33 ± 0.04

UNTARGETED INCREASE   = 2.0 ± 0.1

TARGETED INCREASE   = 0.65 ± 0.05

UV-INDUCED REVERSION FREQUENCY = 1500×10^{-5}

LETHAL HITS (a) = 11.2

* $\pm \Delta R$

express a similar increased reversion of a UV damaged probe as indicated by a deficient targeted increase. A pooled UVEM value one was obtained in the XP variant which was very similar to that observed for AT3BI (Table 13) when infection was delayed 24 hours after cellular irradiation. This suggested that a damage-specific "error-prone" repair system may be defective in these cells when infection was delayed 24 hours following cellular UV-irradiation or alternatively, an inducible error-free system may be activated.

The identification of an untargeted response significantly greater than one (1.6 ± 0.4) which was accompanied by a targeted increase less than one (0.80 ± 0.2 Table 16) suggested that in CRL1162 fibroblasts, adenoviral targeted and untargeted increases were manifestations of separate repair and/or replication systems.

B. IX. UV-enhanced Mutagenesis of Ad5ts36 in Xeroderma Pigmentosum (Group A) Fibroblasts

Cellular hypersensitivity to UV-irradiation has been identified in the case of both classical and variant Xeroderma pigmentosum (XP) fibroblasts as compared to normal fibroblasts. When these UV-induced mutation frequencies were corrected for the decreased cellular survival observed in XP fibroblasts as compared to normals it was determined that XP variant fibroblasts but not the classical XP fibroblasts exhibit UV-induced reversion frequencies greater than normal fibroblasts (Maher and McCormick, 1976; Maher et al, 1976, Maher et al, 1979).

In consideration of these studies and the findings of abnormal expression of UV-irradiated adenovirus in XP strains (Jeeves and

Rainbow, 1983b), XPA (Complementation group A) excision deficient fibroblasts were examined for UVER and UVEM responses using the Ad5ts36 assay similar to that described above for normal, CS and AT fibroblasts. The UV doses used to irradiate either cells or virus were reduced in assays using GM710 (XP25RO) as the host monolayer. The selected dose to XP25RO cells was 0.4 J/m^2 since previous work had established that this dose elicited the maximal UVER of UV-irradiated Ad2 when viral infection was delayed 48 hours after UV to the cells (Jeeves and Rainbow, 1983b). A viral UV dose of 200 J/m^2 was chosen since this will reduce the survival of Ad2 "V" antigen expression to about 10^{-2} in XP25RO similar to the survival of Ad2 observed in normal fibroblasts at a UV dose of about 1.2×10^3 J/m^2, to the infecting virus (Rainbow, 1981). Therefore, the UVEM/UVER progeny data would be comparable to the normal assays which used a higher UV dose to virus but produced similar survival levels. Table 16 contains the typical results for a single progeny assay of XP25RO. The parameters of UV-induced reversion (untargeted increase, targeted increase, UVEM and UVER) for 3 separate experiments are listed in Table 13.

In these progeny assays, the survival of UV-irradiated Ad5ts36 was signficantly reduced in XP25RO as compared to pooled normals (Figure 6). The D_o of progeny production of UV-irradiated Ad5ts36 in XP25RO expressed as a percentage of that obtained on pooled normal strains was about 9 percent, similar to the results obtained previously using Vag as an endpoint (Rainbow, 1981). UV-induced reversion of Ad5ts36 was hypermutable on a per unit dose basis in unirradiated XP25RO fibroblasts as compared to normals (Figure 7). When corrected for progeny survival, a hypermutable UV-induced reversion of Ad5ts36

was not apparent in unirradiated XP25RO as compared to normals (Figure 8). Thus the reduced progeny survival of UV-irradiated virus in unirradiated XP25RO fibroblasts (as compared to normals) appears to be responsible for the hypermutability of UV-irradiated virus when calculated on a per unit dose basis in this excision deficient strain. Since excision proficient normal strains and XP25RO display similar reversion of UV-irradiated virus when calculated per survivor (Figure 8) this suggests that excision repair is an error-free process in agreement with the results of Maher and McCormick (1976) and Lytle et al. (1982).

Table 16 is a typical example of the results obtained from infections of UV-irradiated (0.4 J/m^2) and unirradiated XP25RO fibroblasts with intact and UV-irradiated (200 J/m^2) virus. An untargeted increase of 1.3 $\pm$ 0.1 was found for intact viral replication in UV-irradiated XP25RO fibroblasts. A small targeted increase (1.1 $\pm$ 0.1) was seen in the reversion frequency of UV-irradiated Ad5ts36 progeny from UV-irradiated XP25RO fibroblasts as compared to unirradiated XP25RO monolayer. This resulted in a UVEM less than one (0.83 $\pm$ 0.17), but not significantly less than one. A UVER of 2.2 $\pm$ 0.2 indicated that viral survival was significantly enhanced by preirradiation of the XP25RO monolayers; however, this UVER was not accompanied by significant enhancement of progeny reversion frequencies due to preirradiation of the host cells.

Increasing the UV dose to virus (600 J/m^2) and decreasing the UV dose to XP25RO fibroblasts (0.2 J/m^2) did not significantly change the parameters of Ad5ts36 reversion (Table 13) as compared to that observed in Table 16. Pooling data obtained in assays conducted at similar UV

TABLE 16

Reversion of Ad5ts36 in xeroderma pigmentosum strain
GM710 (XP25R0, passage 31) after 96 hours of lytic growth
at 33°C and titred on HeLa cells at the permissive and
nonpermissive temperatures (24 hour delay in viral
infection following UV to the cells).

| UV Dose J/m^2 | | 39° pfu/ml | 33° pfu/ml | Reversion |
cells	virus	(x 10^3)	(x 10^6)	Frequency* (x 10^{-4})
0	0	8.8	34	2.6 ± 0.1
0.4	0	7.8	23	3.4 ± 0.2
0	200	5.23	2.4	22 ± 1
0.4	200	8.72	3.7	24 ± 1

UVER (33°)   = 2.2 ± 0.2

UVEM   = 0.83 ± 0.17

UNTARGETED INCREASE   = 1.3 ± 0.1

TARGETED INCREASE   = 1.1 ± 0.1

UV-INDUCED REVERSION FREQUENCY = 190 x 10^{-5}

LETHAL HITS (a) = 6.6

* ± ΔR

doses to XP25RO (0.2 J/m^2) and virus (200 J/m^2) resulted in an untargeted increase of 2.0 ± 0.7 and a targeted increase of 1.3 ± 0.3 (means ± 1Σ, Table 13). The resulting UVEM (0.73 ± 0.10) was not accompanied by a UVER (1.4 ± 0.8) significantly greater than one at a UV dose of 0.2 J/m^2 to the cells. Although this dose to cells stimulated an untargeted mutagenesis, it was unable to induce targeted mutagenesis or UVER. This suggests that in XP25RO untargeted mutagenesis can occur independent of targeted mutagenesis and separate mechanisms may be involved in these processes.

B.X. Enhanced Mutagenesis of Intact and UV-irradiated Ad5ts36
In Normal and XP-25RO Fibroblasts Preinfected with Lethally
UV-irradiated HSV-1

Indirect activation of a cellular mutator response has been described after either preinfection of mammalian cells with UV-irradiated viral DNA (Cornelis et al., 1981) or transfection of UV-irradiated DNA from a variety of sources (Dinsart et al., 1984). Since XP25RO fibroblasts are hypersensitive to the lethal effects of direct UV-irradiation, one method of examining UV mutagenesis in these cells (presumably without cellular lethality) is to preinfect fibroblasts with UV-irradiated HSV-1 and examining the mutagenesis of an intact or UV-irradiated viral probe.

A HSV-1 (KOS) preparation was lethally UV-irradiated with 1100 J/m^2 and UV-inactivated HSV-1 was adsorbed onto cells for 60 minutes at room temperature. Unadsorbed virus was removed by washing, cells were refed with complete medium and incubated at 37°C for 24 hours. Cells were treated or left untreated with UV-irradiated HSV-1 and then

infected with either intact or UV-irradiated Ad5ts36 and viral progeny was collected 96 hours later.

Table 17 lists targeted/untargeted increases, UVER and UVEM for the above treatments in GM2674 (normal strain) and XP25RO. Preinfection of GM2674 monolayers with UV-irradiated HSV increased the reversion frequency of progeny from intact virus and resulted in an untargeted increase of 3.6 ± 0.3. Similarly, a large targeted increase (3.8 ± 0.3) was observed for the progeny of UV-damaged (1200 J/m^2) Ad5ts36 infecting preinfected monolayers. An indirectly induced enhancement of mutagenesis (ratio of targeted to untargeted increase) of 1.1 ± 0.2 was observed in the absence of ER (0.35 ± 0.03). It appears that exogenously damaged DNA (UV'D HSV) may be incapable of inducing UVER but was an efficient inducer of untargeted and targeted mutagenesis in normal strain GM2674. This agrees with the work of Cornelis et al. (1981) and Dinsart et al. (1984) that suggests indirect induction induces a cellular mutator responsible for untargeted mutagenesis.

The previously described procedure using UV-irradiated HSV-1 as an "inducing" agent was applied to XP25RO to examine adenovirus progeny UVER and UVEM responses independent of cellular lethality. "Induced" or uninduced (control) XP25RO fibroblasts were infected with either unirradiated or UV-irradiated (200 J/m^2) Ad5ts36, 24 hours after infection with lethally UV-irradiated HSV-1. Table 17 lists the targeted/untargeted increases, UVER and UVEM for UV-irradiated Ad5ts36 in confluent XP25RO fibroblasts.

Indirect UV-induction of XP25RO fibroblasts with UV'D HSV resulted in the enhanced reversion of both intact and UV-irradiated

TABLE 17

Enhanced reversion of intact and UV-irradiated Ad5ts36 in normal and
XP25RO strains, preinfected with lethally UV-irradiated HSV-1.
Indirect induction of fibroblasts was 24 hours prior to Ad5ts36
infection. After 96 hours of lytic growth at 33°C, viral progeny
was titred on HeLa cells at the permissive and nonpermissive temperatures.

NORMAL (GM2674)			XP25RO		
UV'D HSV	UV Dose to Virus (J/m^2)	Reversion Frequency $(x\ 10^{-5})$*	UV'D HSV	UV Dose to Virus (J/m^2)	Reversion Frequency $(x\ 10^{-5})$*
-	0	2.5 ± 0.1	-	0	7.2 ± 0.2
+	0	8.8 ± 0.3	+	0	11 ± 0.2
-	1200	130 ± 5	-	200	53 ± 2
+	1200	500 ± 20	+	200	97 ± 2

Enhanced Mutagenesis   = 3.6 ± 0.3   = 1.6 ± 0.1
of Intact virus
(Untargeted Increase)

Enhanced Mutagenesis   = 3.8 ± 0.3   = 1.8 ± 0.1
of UV-irradiated Virus
(Targeted Increase)

Indirectly Induced   = 1.1 ± 0.2   = 1.1 ± 0.1
Enhanced Mutagenesis

Enhanced Reactivation   = 0.35 ± 0.03   = 1.3 ± 0.1

UV-induced RF   = 129×10^{-5} = 46×10^{-5}

Lethal Hits   = 8.6   = 6.3

Ad5ts36. The enhanced mutagenesis observed for intact virus (untargeted increase 1.6 ± 0.1) was similar to that for UV-irradiated virus (targeted increase 1.8 ± 0.1). Therefore, the indirectly induced enhancement of mutagenesis (1.1 ± 0.1) indicated that an induced cellular mutator was equally active for intact or UV-irradiated virus. ER of UV-irradiated adenovirus was only slightly enhanced (1.3 ± 0.1) in XP25RO preinfected with UV'D HSV.

Comparison of direct vs indirect UV-induction in GM2674 and XP25RO suggests that the nature of the signalling mechanism may be different. Direct UV irradiation of GM2674 or XP25RO (Table 17) followed by Ad5ts36 infection 24 hours later resulted in differences in the magnitude of the untargeted vs the targeted increase in both cell strains. Since indirect activation equally activated untargeted vs targeted increases in both GM2674 and XP25RO, this suggests a difference in the nature of the signal(s) responsible for the mutator response. It is possible that the UV-damaged HSV may code for (or induce) the mutator function directly. Although no viable HSV progeny were detected from GM2674 or XP25RO monolayers infected with UV-irradiated HSV alone, DNA repair may allow the expression of a small HSV "mediator", protein responsible for the EM effect.

C. Comparison of UV-Induced Ad5ts36 Progeny Reversion in Normal and Repair-deficient Human Fibroblasts

Data utilizing Ad5ts36 was used to examine the kinetics of induction of progeny reversion in unirradiated cells as a function of progeny survival at 33°C. From the progeny surviving fractions the number of lethal hits "a" was calculated using the Poisson distribution

such that $SF = e^{-a}$ and $a = - \ln SF$. Table 18 lists the lethal hits and UV-induced reversion frequencies calculated for the progeny resulting from infections of unirradiated and UV-irradiated virus in unirradiated fibroblasts. These values have previously been plotted to demonstrate the kinetics of UV-induced progeny reversion as a function of viral lethal hits in unirradiated fibroblasts (Figure 8) and incorporates the data of Table 2.

A plot of UV-induced reversion frequency (x 10^{-4}, linear scale) versus the progeny lethal hits in pooled unirradiated fibroblasts indicated that initially the UV-induced reversion frequency followed a linear function until approximately 8-9 lethal hits then rapidly increased resembling a "power" function which, when transformed was of the form: $y = b (x)^n$, (10) where

y = the UV-induced reversion frequency

b = the interception of the line with the

ordinate at $x = 1$

x = the "dose" or, in the above

example, the "lethal hits"

n = the slope of the line, or the average

number of "hits" required to produce

a mutagenic (reversion) event

Above equation from Kolmark and Kilbey ('1968).

Transformation of the data of Table 18 by plotting the log (UV-induced reversion frequency) versus the log "lethal hits" resulted in a straight line (Figure 7) of the form $y = b (x)^n$. Using linear regression analysis, a straight line was fitted to the data of Figure 8

which demonstrated a dose dependent (a varies directly with UV dose) increase in UV-induced reversion of Ad5ts36 progeny. Since this figure used a number of separate viral UV-irradiations (11 of 30 were at a UV dose of 1200 J/m^2 to the virus), it was more significant in terms of linear regression to plot dose as a function of survival.

Figure 8 combines data for both normal human fibroblasts (open symbols) and repair deficient fibroblasts (closed symbols). From an examination of the points, it was not possible to identify any human strains that were constitutively hypermutable when plotted as a function of lethal hits among UV-irradiated adenovirus progeny. As a comparison the Ad5ts36 data in CRL1221 (Figure 5) were plotted such that log UV-induced reversion frequency of viral progeny vs the log lethal hits (for progeny) produced a similar linear response (data not shown). The resulting slope obtained using CRL1221 normal host monolayers was not significantly different from the slope obtained by combining normal and repair deficient human fibroblasts from individual progeny assays (Figure 8).

The effects of UV-irradiation of the host fibroblast monolayers on the kinetics of expression of UV-induced reversion can be plotted in a manner analogous to Figure 8. A plot of the log UV-induced reversion frequency of viral progeny versus the log number of viral lethal hits from induced fibroblasts (24 hours before infection) also produced a linear responses (data not shown) such that an increased slope (not significantly greater than unirradiated) was obtained. The existence of an error-prone repair system active in preirradiated normal human fibroblasts suggested by the results of Table 5 indicated that normal strains show a small but significant UVEM thus giving

– evidence for an error-prone repair process. In order to illustrate this phenomenon further, the progeny reversion and survival data from assays where infections were delayed 24 hours after cellular UV were combined to plot UV-induced reversion frequency as a function of lethal hits in both unirradiated and UV-irradiated cells.

If the UV-induced reversion frequencies obtained in unirradiated fibroblasts (data from Figure 7) or UV-induced fibroblasts are plotted vs progeny lethal hits on a linear – linear axis, two separate curves resulted (Figure 9). Data from UV-induced fibroblasts produced a linear UV-induced reversion frequency response until an average progeny damage level of 5 to 6 lethal hits accumulated followed by a sharp increase in UV-induced reversion frequency resembling a power function. Unirradiated fibroblasts produced a linear UV-induced reversion frequency response until approximately an average of 8 lethal hits accumulated among the progeny. This was followed by a similarly sharp increase in the UV-induced reversion frequency resembling the power function increase observed in UV-induced fibroblasts. The sharp increase in UV-induced reversion frequency occurred after less accumulated lethal damage among progeny obtained from UV-induced fibroblasts when compared to unirradiated fibroblasts.

Furthermore, this suggests that the ability to detect enhanced mutagenesis in UV-irradiated human cells is dependent on the viral damage levels. Only when the UV-damage level is greater than that necesary to produce 5–6 lethal hits will UVEM phenomena be observed.

A UVER value of 3.4 ± 0.8 for UV-irradiated adenovirus in pooled normal strains infected 24 hours after cellular irradiation showed that preirradiation of cells resulted in an increased progeny

survival mediated by an increase in the repair of viral DNA lesions. This resulted in a reduction in the number of lethal hits among the progeny obtained from UV-irradiated cells. In Figure 9, this is depicted by a leftward shift of data points obtained from UV-irradiated as compared to unirradiated fibroblasts. Furthermore, reduction in the UV-dose will also reduce the number of lethal hits for viral progeny obtained from unirradiated or UV-irradiated fibroblasts (Figure 9). Such a reduction in the number of lethal hits from changes in the UV-dose and/or differences in constitutive DNA repair capability results in a reduction in the progeny reversion frequencies. This shifts data points towards the origin (y-intercept) of Figure 9 along the depicted curves. However, a reduction in the number of lethal hits as a result of pre-irradiation of the cells alone (due to the existence of UVER) is not accompanied by a reduction in progeny UV-induced reversion frequency. This suggests that the induced repair mechanism (UVER) which renders the lesions non-lethal is not error-free. Moreover, this implies that experiments displaying little or no UVEM (UVEM=1) but are accompanied by a high UVER (1) indicate the presence of error-prone repair. If UVER was an error-free system, decreases in progeny lethal hits (as the result of UVER) would be concomitant with decreases in progeny UV-induced reversion frequencies in UV-irradiated as compared, to unirradiated fibroblast hosts. Since conventional UVEM calculations (equations 1 and 4) do not incorporate correction for enhanced viral survival (UVER) in UV-irradiated as compared to unirradiated cells, an alternate calculation of UVEM based on the results of Figure 9 may be more appropriate). Alternatively UVEM may be defined as:

$$UVEM = \frac{(RF\ of\ UV\text{-}irradiated\ Virus\ in\ UV\text{-}irradiated\ Cells) - (RF\ of\ Intact\ Virus\ in\ UV\text{-}irradiated\ Cells)}{(RF\ Of\ UV\text{-}irradiated\ Virus\ in\ Control\ Cells) - (RF\ of\ Intact\ Virus\ in\ Control\ Cells)}$$

$$= \frac{UV\text{-}Induced\ Reversion\ Frequency\ Obtained\ from\ UV\text{-}Induced\ Cells}{UV\text{-}Induced\ Reversion\ Frequency\ in\ Control\ Cells\ (Unirradiated)} \tag{11}$$

This equation measures the fold differences (enhancement) observed between the progeny reversion frequencies of UV-induced and control fibroblasts in Figure 9. This previous equation (11) does not correct for the enhanced survival (UVER) observed in UV-irradiated as compared to unirradiated fibroblasts. As a result UVEM must be corrected for survival differences as follows:

$$UVEM = \frac{UV\text{-}Induced\ Reversion\ Frequency\ per\ Lethal\ Hit\ in\ UV\text{-}Irradiated\ Cells}{UV\text{-}Induced\ Reversion\ Frequency\ per\ Lethal\ Hit\ in\ Unirradiated\ Cells} \tag{12}$$

$$= \frac{\dfrac{UV\text{-}Induced\ RF}{Progeny\ Lethal\ Hits}\ (UV\text{-}irradiated\ cells)}{\dfrac{UV\text{-}Induced\ RF}{Progeny\ Lethal\ Hits}\ (Unirradiated\ cells)}$$

Table 18 contains the UVEM values calculated using either equation 1 ($UVEM_1$) or equation 12 ($UVEM_2$) for Ad5ts36 progeny assays in normal and repair deficient fibroblasts infected 24 hours following cellular UV-irradiation. The calculated $UVEM_2$ values were larger than the $UVEM_1$ values (18 of 20 progeny experiments). Data from pooled

normal fibroblasts infected 24 hours after cellular UV-induction produced a mean ($\pm$1SE) $UVEM_2$ value of 2.4 $\pm$ 0.4 compared to a mean $UVEM_1$ value of 1.4 $\pm$ 0.2 (Table 5). This indicates that calculations utilizing equation 12 ($UVEM_2$) are significantly greater than those using equation 1 ($UVEM_1$).

D.I. UV-enhanced Reactivation of Vag Synthesis for UV-irradiated Adenovirus in AT Fibroblasts

Previous results from this laboratory (Jeeves and Rainbow, 1983d) have shown an aberration of gamma-ray enhanced reactivation of adenovirus Vag synthesis in AT strains. Since the results of Table 13 indicated some aberration of UVEM in AT strains as compared to normal fibroblasts, it was considered of interest to examine UVER of adenovirus Vag synthesis in AT strains.

D.I.a. Immediate Viral Infection

Normal human fibroblast strains CRL1221 and GM969 and AT strains AT3BI and GM1588 were grown to confluency upon 8-well chamber slides. Fibroblasts were UV-irradiated immediately, 24 or 48 hours prior to infection with intact or UV-irradiated Ad2. The cellular UV-irraditions (0, 5, 10, 15, J/m^2) were staggered such that viral infection was at the same time for the different delays of viral infection following cellular UV-irradiation. Typical results for infection of cells immediately after UV are given in Figure 10 and pooled UVER values for a number of experiments are shown in Figure 11 (upper panels).

In Figures 10 and 11 it can be seen that the results for the

two normal controls were similar to that observed by Jeeves and Rainbow, 1983a. In particular the intact virus produced more Vag positive cells following cellular UV-irradiation as compared to unirradiated normal cells. UV-irradiated virus also produced larger relative Vag formation following cellular UV-irradiation when compared to unirradiated normal fibroblasts. This resulted in reactivation factors greater than one at all UV doses to the normal fibroblasts. However UVER was much reduced in AT fibroblast strains tested at UV doses greater than 5 J/m^2 to the cells (Figures 10 and 11).

D.1.b. 24 Hours Delayed Viral Infection

Typical results obtained after infection of cell monolayers 24 hours following cellular UV-irradiation are shown in Figure 12 and pooled results for a number of experiments are given in Figure 11 (lower panel). It can be seen that although the spread of UVER values for normals was similar to that for immediate infection, the UVER values for AT strains had increased compared to those for immediate infection. Pooled results showed that UVER values for AT strains could not be distinguished from normals after a 24 hour delayed viral infection.

D.1.c. 48 Hours Delayed Viral Infection

Figure 13 contains results obtained when viral infection was delayed 48 hours following cellular UV-irradiation. In general UVER values decreased in normals as compared to immediate or 24 hour delays as described previously (Jeeves and Rainbow, 1983a). Normal strains were not distinguishable from AT which has been shown in Figure 13.

D.II. Time Course of Expression of UVER

Results of the previous section indicated a variation in the time course expression of UVER in AT as compared to normal strains. In order to illustrate this difference Figure 14 shows the UVER factors for a fixed UV dose to the cells (15 J/m^2) as a function of time between cellular irradiation and viral infection. It can be clearly seen that the AT strains tested show a markedly reduced UVER for immediate infection (UVER factor less than 1). The UVER value for AT strains increased as a function of time between UV to the cells and subsequent viral infection such that UVER values for AT strains is greater than 1 by 12 hours and approaches normal levels at 24 hours.

This data indicates an aberration of UVER in AT cells as compared to normals which was most evident when there was no infection delay after cellular UV-irradiation. Abnormal UVEM was also observed in AT3BI when infection was delayed 24 hours post cellular irradiation suggesting that both processes may result from the DNA repair defect associated with AT fibroblasts.

D.III Ad2 Direct Plaque Expression of UVER in Normal and AT

Fibroblast Strains UV-Irradiated 24 hours Before Viral Infection

Ad2 viral plaque formation represents an event farther removed from viral DNA repair and replication than "V" ag formation. A larger functional proportion of the Ad viral genome is required for the production of infective progeny as compared to viral "V" antigen production which depends on the viral expression of late capsid proteins such as hexon and fibre. These proteins are subsequently

Figure 9

The UV-induced reversion frequency of Ad5ts36 progeny obtained
from unirradiated (open symbols) or UV-preirradiated[*] (closed symbols)
human fibroblasts as a function of viral progeny lethal hits.

◯ CRL1221

◇ A2

▽ GM2674

⬡ GM969

▢ GM288

⬦ GM2838

⬠ XP25R0

△ AT3BI

⬰ CRL1162

* cells were UV-irradiated 24 hours prior to viral infections, data
  obtained from Table 18.

† UV-irradiation of cells resulted in UVEM values significantly less
  than one (AT3BI and CRL1162)

Figure 9
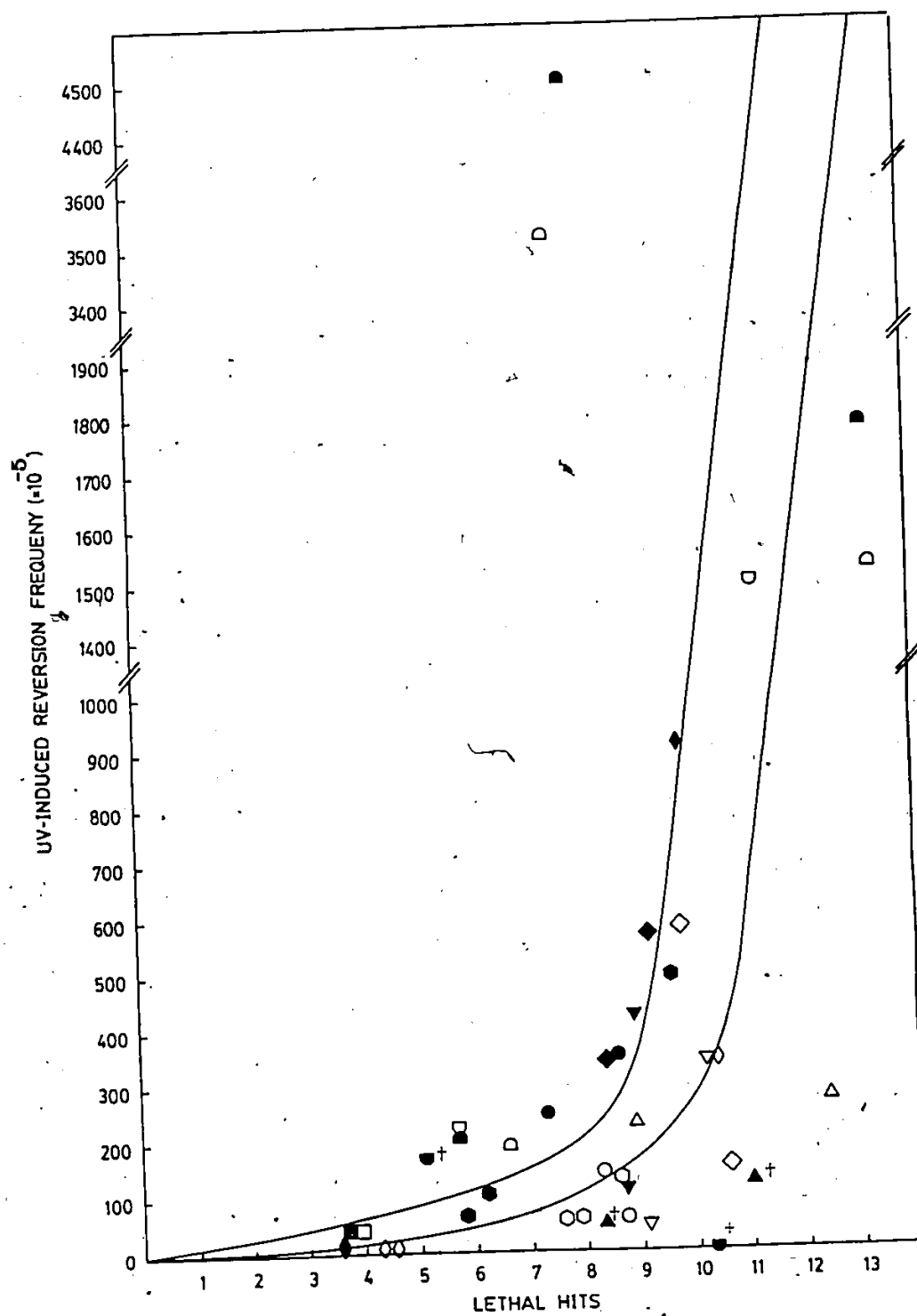
T A B L E   1 8

UVEM OF UV-IRRADIATED AD5TS36 CALCULATED AS THE RATIO OF UV-INDUCED RF PER LETHAL HIT IN UV-IRRADIATED CELLS VS. THE UV-INDUCED RF PER LETHAL HIT IN UNIRRADIATED CELLS. VIRAL INFECTIONS WERE DELAYED 24 HOURS AFTER CELLULAR IRRADIATION.

	A UV-Induced RF (uninduced cells, x 10^{-5})	a_1 (Lethal Hits)	$\frac{A}{a_1}$	B UV-Induced RF (UV'D cells, x 10^{-5})	a_2 (Lethal Hits)	$\frac{B}{a_2}$	UVEM$_1$ *	UVEM$_2$
NORMALS								
A2	149	10.6	14	338	8.4	40	1.2	2.9
A2	580	9.8	59	565	9.2	61	0.76	1.0
CRL1221	140	8.3	17	350	8.6	41	0.96	2.4
CRL1221	56.8	8.7	6.5	245	7.3	34	1.5	5.2
GM2674	44.3	9.1	4.8	108	8.7	12	2.1	2.5
GM2674	340	10.2	33	420	8.9	47	1.1	1.4
GM969	58.5	7.9	7.4	101	6.2	16	0.95	2.2
GM969	55.2	7.6	7.3	63.1	5.8	11	1.8	1.5
GM2674	128	8.6	15	491	9.6	51	1.1	3.4
GM288	39	3.8	10	42	3.7	11	0.82	1.1
COCKAYNE'S SYNDROME								
GM2838	6.95	4.3	1.6	9.4	3.6	2.6	1.2	1.6
GM2838	9.0	4.6	2.0	15.4	3.6	4.2	1.0	2.1
GM2838	338	10.4	32.5	914	9.8	93	1.1	2.9
ATAXIA TELANGIECTASIA								
AT3BI	270	12.4	22	120	11	11	0.26	0.50
AT3BI	229	8.9	26	50.3	8.3	6.1	0.17	0.23
XERODERMA PIGMENTOSUM								
XP25RO	190	6.6	29	206	5.7	36	0.83	1.2
XP25RO	3,520	7.6	463	4,500	8.0	562	0.62	1.2
XP25RO	1,530	13.3	115	1,780	13.2	135	0.80	1.2
CRL1162	219	5.7	38	168	5.1	33	0.72	0.87
CRL1162	1,500	11.2	134	1	10.3	0.10	0.33	-

Key:

$\text{UVEM}_1 = \dfrac{\text{targeted increase}}{\text{untargeted increase}}$

$\text{UVEM}_2 = \dfrac{B/a_2}{A/a_1}$

$a_1 = \text{SF} = e^{-a_1}$ (calculated for untargeted cells)

$a_2 = \text{SF} = e^{-a_2}$ (calculated for UV-irradiated cells)

* Data from progeny assays (Tables 5, 13)

Figure 10

The relative "V" ag formation for intact and UV-irradiated Ad 2 in normal and AT human fibroblast strains UV-irradiated immediately before viral infection.

○ CRL1221   (passage 20)
⬡ GM969   (passage 20)
△ AT3BI   (passage 16)
▽ GM1588   (passage 20)

UPPER PANEL: Upper Curves: relative "V" ag formation for
                            intact (unirradiated virus)

              Lower Curves: relative "V" ag formation for
                            UV-irradiated virus (1200 J/m^2)

BOTTOM PANEL: UVER factors (closed symbols)

Figure 10
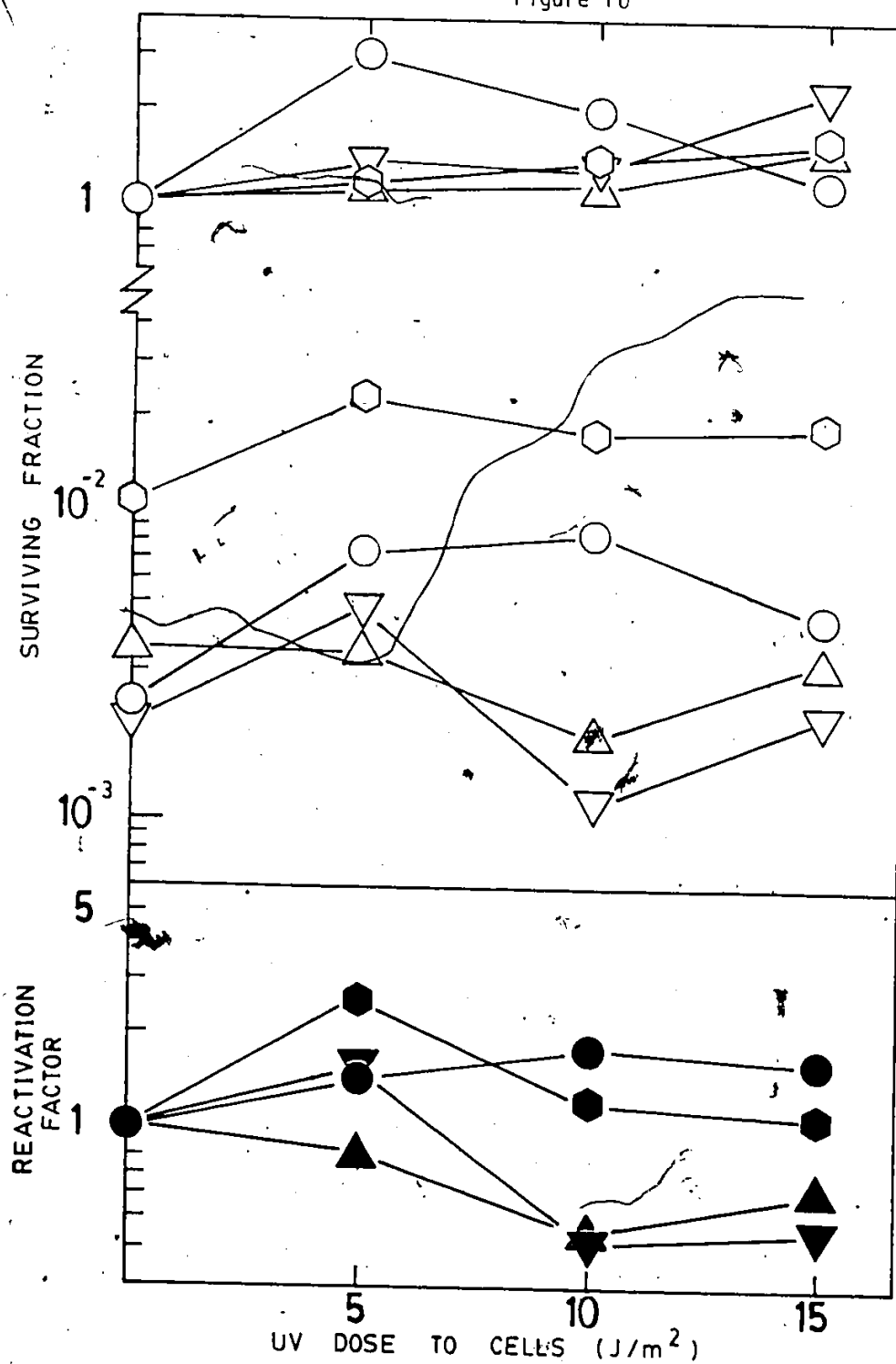
Figure 11

The UVER of UV-irradiated (1200 J/m^2) Ad 2 in pooled normal and AT fibroblast strains as a function of UV-dose to the cells.

◯ CRL1221*

⬡ GM969

◇ Mean (normals)

▲ AT3BI

▼ GM1588

◆ Mean (AT strains)

UPPER PANEL: Cells UV-irradiated immediately before
              viral infection

BOTTOM PANEL: Cells UV-irradiated 24 hours prior to
              viral infection

* data obtained from this study Figures 10,12 and the data of Rainbow
  et al, 1983 (see Figure 14)

Figure 11
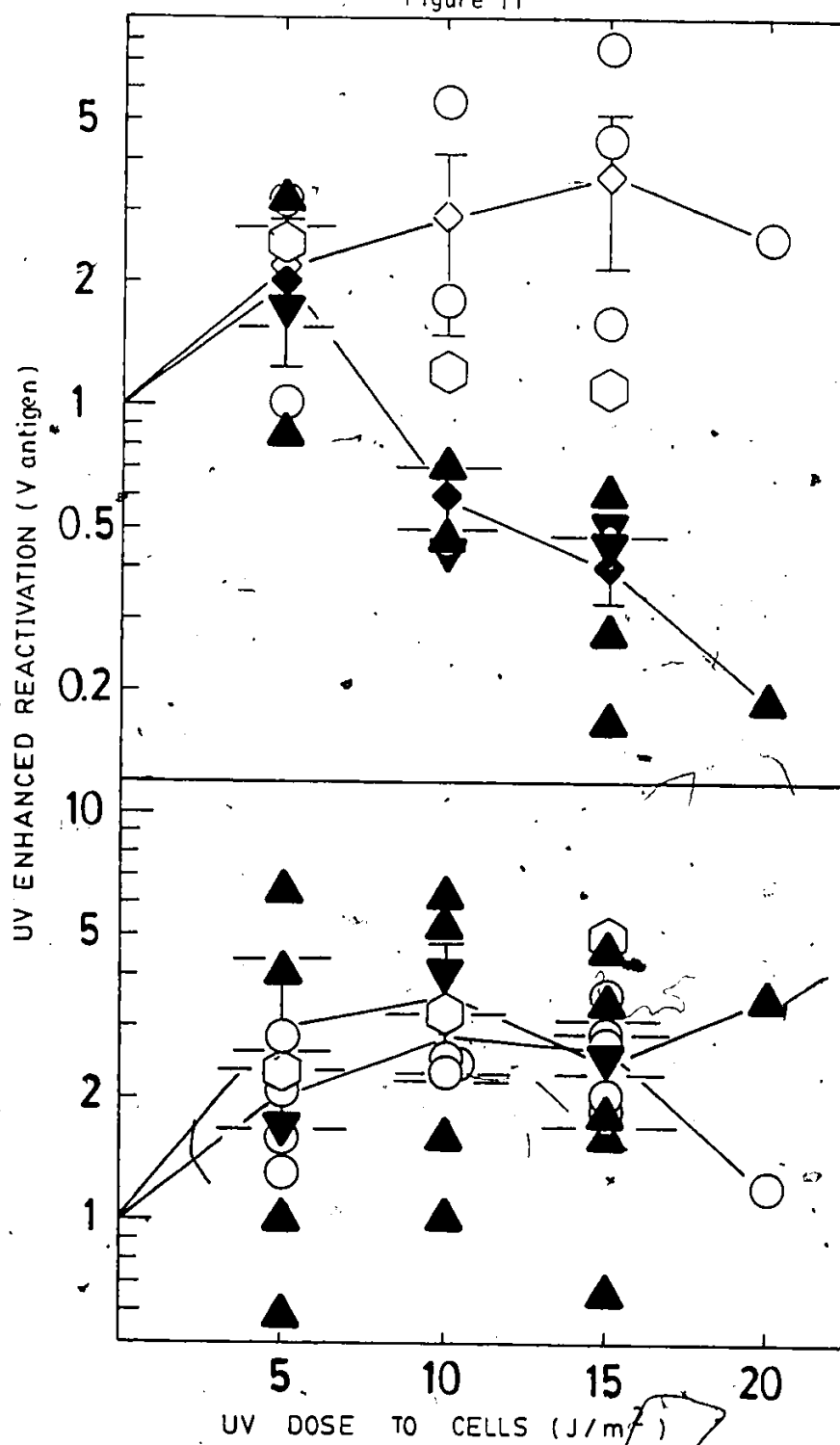
Figure 12

The relative "V" ag formation for intact and UV-irradiated Ad 2
in normal and AT human fibroblast strains UV-irradiated 24 hours before
viral infection.

◯ CRL1221 (passage 20)

⬡ GM969 (passage 20)

△ AT3BI (passage 16)

▽ GM1588 (passage 20)

UPPER PANEL: Upper Curves: relative "V" ag formation for

intact (unirradiated virus)

Lower Curves: relative "V" ag formation for

UV-irradiated virus (1200 J/m^2)

BOTTOM PANEL: UVER factors

Figure 12
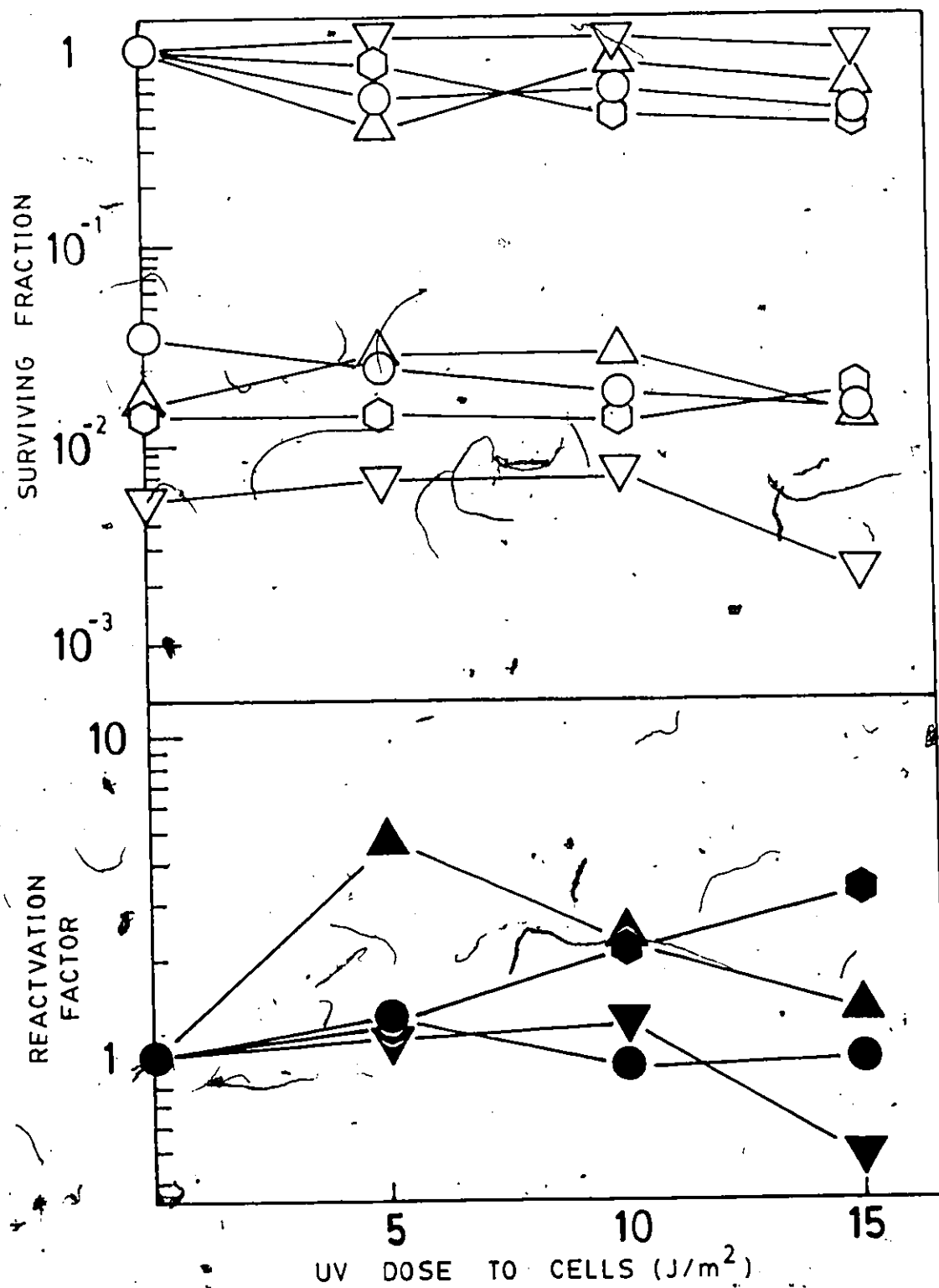
Figure 13 .

The relative "V" ag formation for unirradiated and UV-irradiated Ad 2 in normal and AT human fibroblast strains UV-irradiated 48 hours before viral infection.

⭘ CRL1221 (passage 20)

⬡ GM969 (passage 20)

△ AT3BI (passage 16)

▽ GM1588 (passage 20)

UPPER PANEL: Upper Curves: relative "V" ag formation for
intact (unirradiated virus)

Lower Curves: relative "V" ag formation for
UV-irradiated virus (1200 J/m^2)

BOTTOM PANEL: UVER factors

Figure 13 139
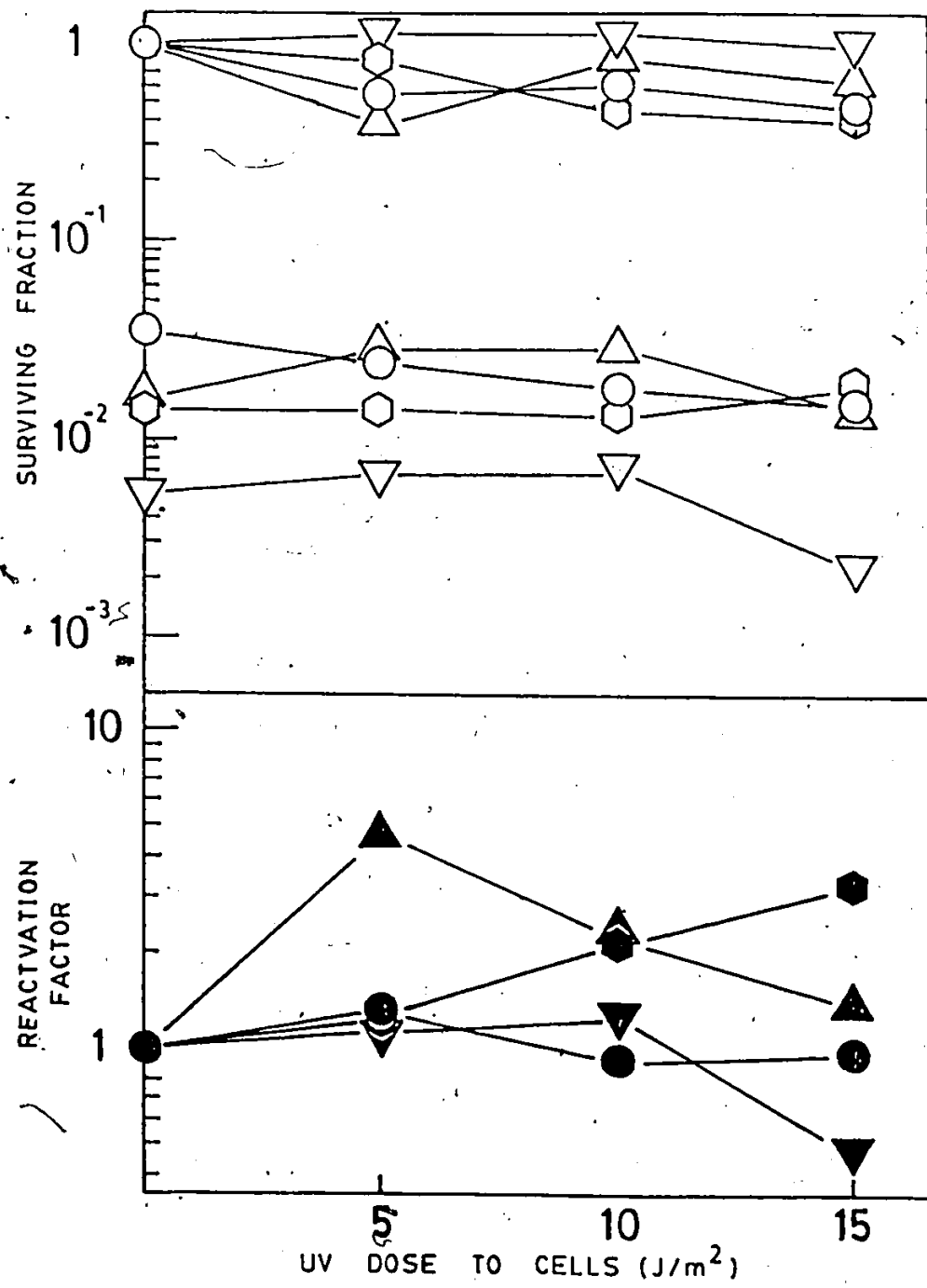
Figure 13

Figure 14

UV-enhanced reactivation of relative "V" antigen production of UV'D Ad2 in pooled, UV-irradiated (15 J/m^2), normal and AT fibroblast monolayers as a function of infection delay (in hours) following UV to the cells.

Pooled normal strains:

○ CRL1221 (passage 17)+

○ CRL1221 (passage 17)*

● ○ CRL1221 (passage 20)*

○ CRL1221 (passage 20)+

⬡ GM969 (passage 20)*

□ GM288 (passage 17)+

Pooled AT strains:

▲ AT3BI (passage 16)*

▲ AT3BI (passage 15)*

▲ ▲ AT3BI (passage 15)+

▲ AT3BI (passage 20)+

▼ GM1588 (passage 20)*

▼ GM1588 (passage 15)+

* data obtained from this study Figures 19, 20, 21, 22, 23

+ data of Rainbow et al, 1983

Figure 14
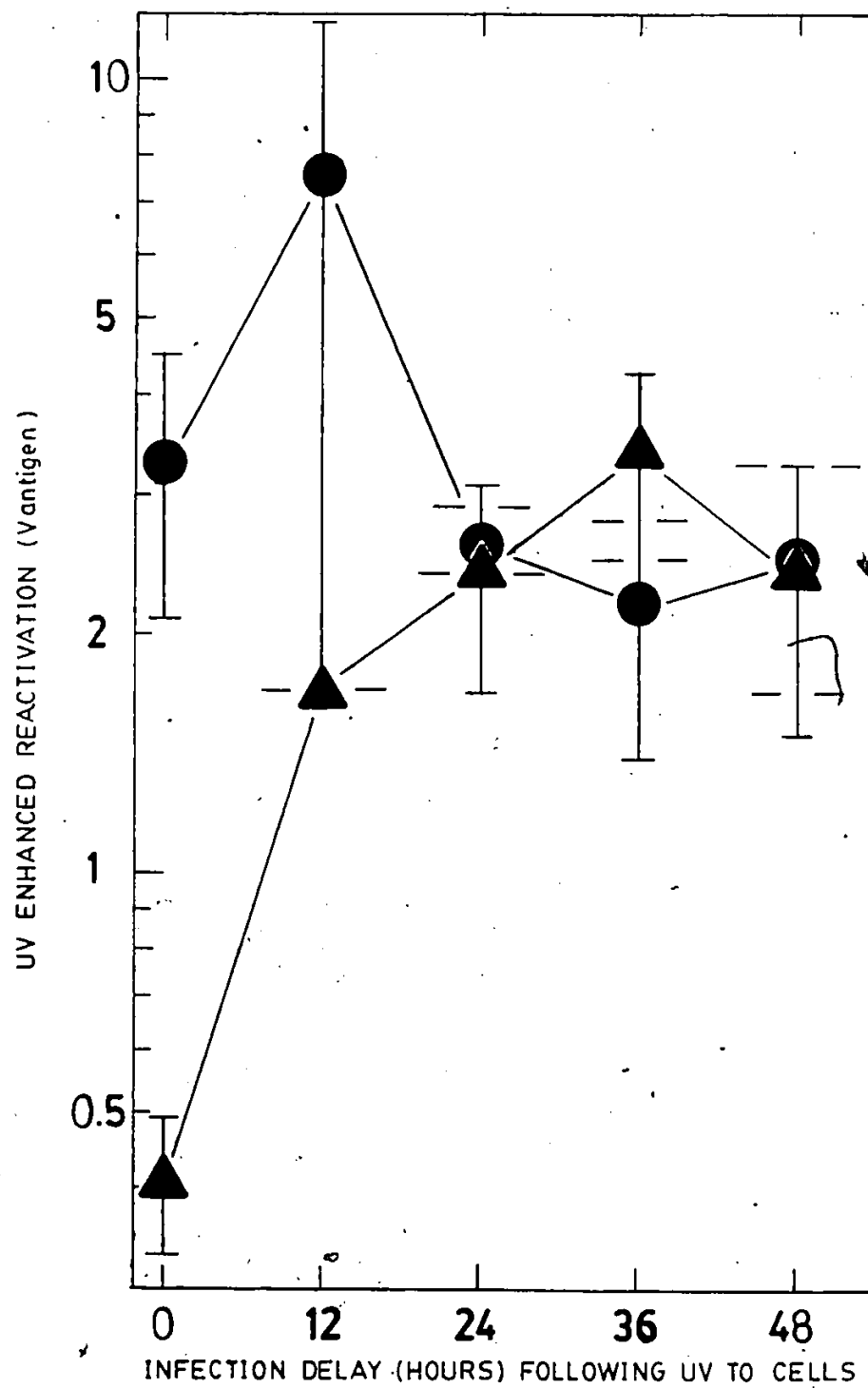
detected by immunofluorescent staining in the "V" ag assay.

Since survival of Vag synthesis does not require in all likelihood high fidelity of DNA repair, it is thought this may be a sensitive assay for error-prone repair. The apparently normal UVER of Vag formation for UV-irradiated adenovirus in AT as compared to normal fibroblasts when viral infections occurred 24 hours after cellular irradiation prompted an examination of AT and normal fibroblasts for UVER using an assay (direct plaque formation) that requires a high fidelity of repair.

Figure 15 illustrates the survival of intact (top panel, upper curves) and UV-irradiated Ad2 (top panel, lower curves). UV-irradiation of the cells inhibited the plaque formation of intact virus in both CRL1221 and AT3BI. UV-irradiation of the virus reduced the plaque SF to 2.3×10^{-3} and 4.1×10^{-3} for unirradiated AT3BI and CRL1221 respectively. This was significantly smaller than the SF's observed using the "V" antigen assay under similar conditions (Figure 15). The plaque forming ability of UV-irradiated virus was inhibited in CRL1221 as UV-dose to the cell monolayers increased. The plaque forming ability of UV-irradiated Ad2 in AT3BI increased slightly upon cellular UV-irradiation (Figure 15, top panel, bottom curves). At a dose of 10 or 15 J/m^2, the UV-irradiated Ad2 plaque survival was greater in AT3BI than that observed in CRL1221.

The UV enhanced reactivation of plaque forming ability was greater in AT3BI than that observed in CRL1221 although this increase was significant only at a dose of 10 J/m^2 to the cells (Figure 15). The increase in direct plaquing UVER values of Ad2 on AT3BI versus CRL1221 may reflect the hypomutable nature or "error-free" repair of

Figure 15

UVER of the relative plaque forming ability for intact and UV-irradiated Ad2 in normal (CRL1221) and ataxia telangiectasia (AT3BI) fibroblast strains. Cell monolayers were infected 24 hours after cellular UV-irradiation.

○ CRL1221

△ AT3BI

TOP PANEL: Upper Curves: relative plaque forming ability
                             for intact (unirradiated virus)

             Lower Curves: relative plaque forming ability
                             for UV-irradiated (1200 J/m^2)

BOTTOM PANEL: UVER factors

Figure 15
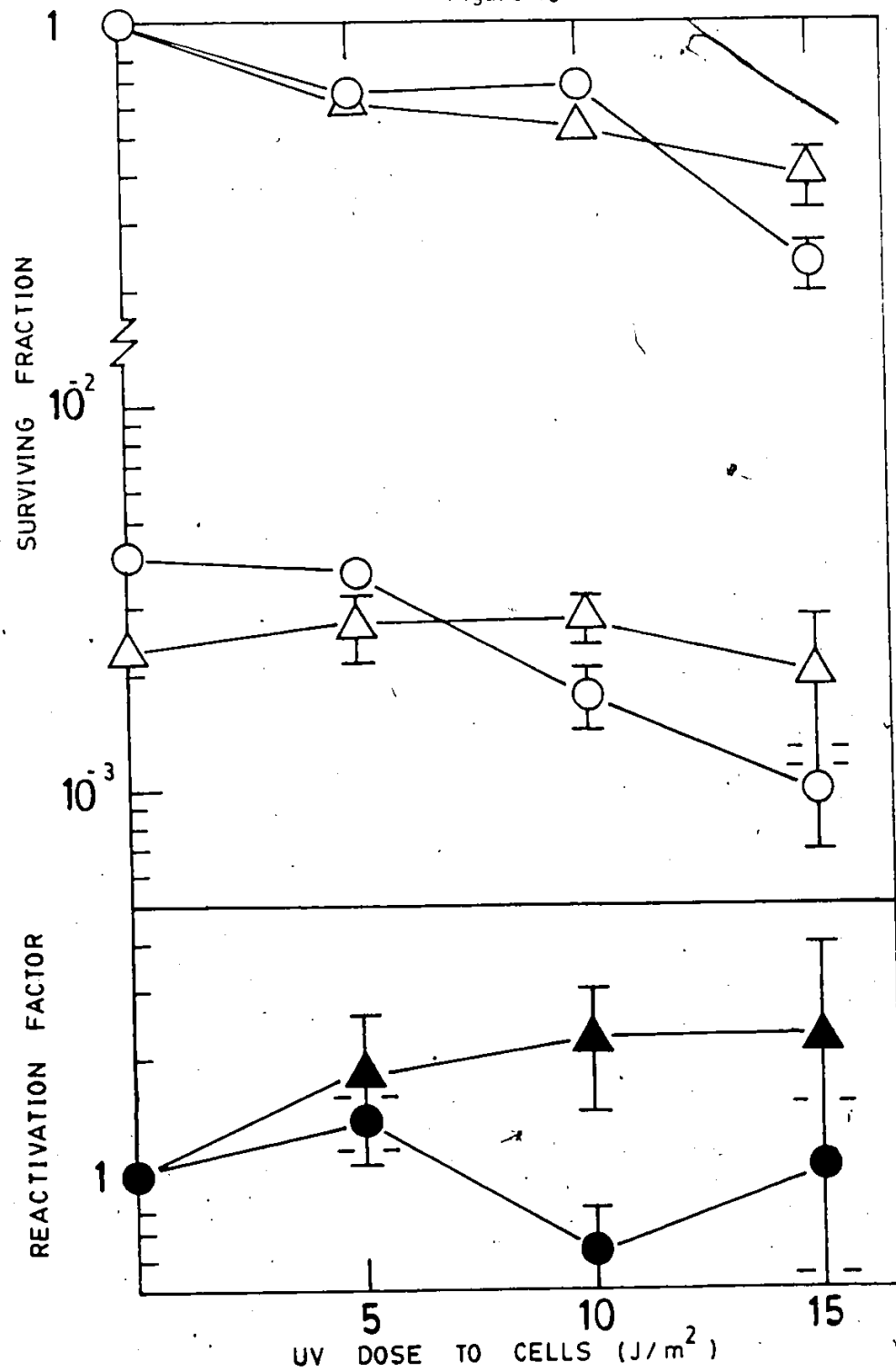
the Ad2 replication in AT.

E.I. DNA Damage and Biological Expression of Human Adenovirus Following Gamma Irradiation at 0°C; Double Strand DNA Breakage

Previous work from this laboratory has been carried out on the HCR of human fibroblast strains with regard to their ability to reactivate Ad2 virus gamma-irradiated at -75°C. Under those irradiation conditions, radiation induced DNA strand breakage has been correlated with inactivation of the biological functions of the virus (Rainbow and Mak, 1972). The enhanced reactivation and HCR of gamma-irradiated Ad2 has also been examined in both normal and repair deficient human fibroblasts such as XP and CS (Rainbow and Howes, 1979; Jeeves and Rainbow, 1979; Jeeves and Rainbow, 1983a; b; c). The survival of Ad2 gamma-irradiated at -75°C (in the frozen state) was significantly reduced in XP as compared to normal fibroblast strains (Rainbow and Howes, 1979) such that the D_o for the survival of "V" ag expression of gamma-irradiated Ad2 in XP25RO (complementation group A) was about 65% of that obtained in normal strains. Similarly, reduced levels of HCR have been reported by other laboratories in XP following gamma-irradiation of Ad2 at -75°C (Brown et al, 1980). Thus gamma-irradiation of Ad2 at -75°C results in DNA damage that is poorly repaired in XP as compared to normal.

Since it is unlikely that the frozen irradiation conditions previously used to irradiate Ad2 would exist for biological systems in the natural environment, it was considered of interest to examine the molecular DNA damage and the subsequent consequences on biological expression of Ad2 following gamma-irradiation of the virus under liquid

conditions. Ad2 was gamma-irradiated in the liquid state (on ice at 0°C) suspended in tris buffered saline containing 20 percent glycerol and subsequently assayed for DNA strand breakage (using sucrose gradient sedimentation). The observed DNA breakage was then correlated with the survival of several viral functions including adsorption and plaquing on human KB cells and "V" ag formation in both normal and XP fibroblasts.

Gamma-irradiated virus containing ^{3}H-DNA together with unirradiated marker virus, containing ^{14}C-DNA were lysed with pronase and sodium dodecyl sulphate (SDS). Radioactivity profiles obtained from the neutral sucrose gradients as shown in Figure 16, typically display no shifting of the peaks when comparing the irradiated and non-irradiated DNA. The radioactivity peak of the unirradiated marker ^{14}C labelled viral DNA was normalized to the peak of the ^{3}H labelled viral DNA for each gradient. The ^{3}H graph profile sedimented over the ^{14}C marker profile after the normalization. It was assumed that the ^{14}C sedimenting marker represented those DNA molecules with no double-strand breaks. The fraction of DS molecules that were unbroken was calculated by:

$$\frac{\text{the area under the } ^{14}\text{C peak}}{\text{the area under the } ^3\text{H peak}} = \text{Fraction of molecules unbroken}$$

The areas were estimated by cutting out the graphs and weighing them on an analytical balance. Typical values obtained from these fractions are shown in Table 19. The average number of double-strand breaks per molecule was calculated from the Poisson distribution as follows:

Figure 16

Radioactivity profiles of Ad 2 DNA sedimented on neutral sucrose gradients.

TOP PANEL (a): ^{3}H-labelled, unirradiated Ad 2 viral
                  DNA (○) and unirradiated ^{14}C-labelled
                  marker Ad 2 DNA (△)

BOTTOM PANEL (b): Shows ^{3}H-labelled, gamma-irradiated
                   (0.58 Mrads) Ad 2 viral DNA (○) and
                   unirradiated ^{14}C-labelled marker
                   Ad 2 DNA (△)

Figure 16
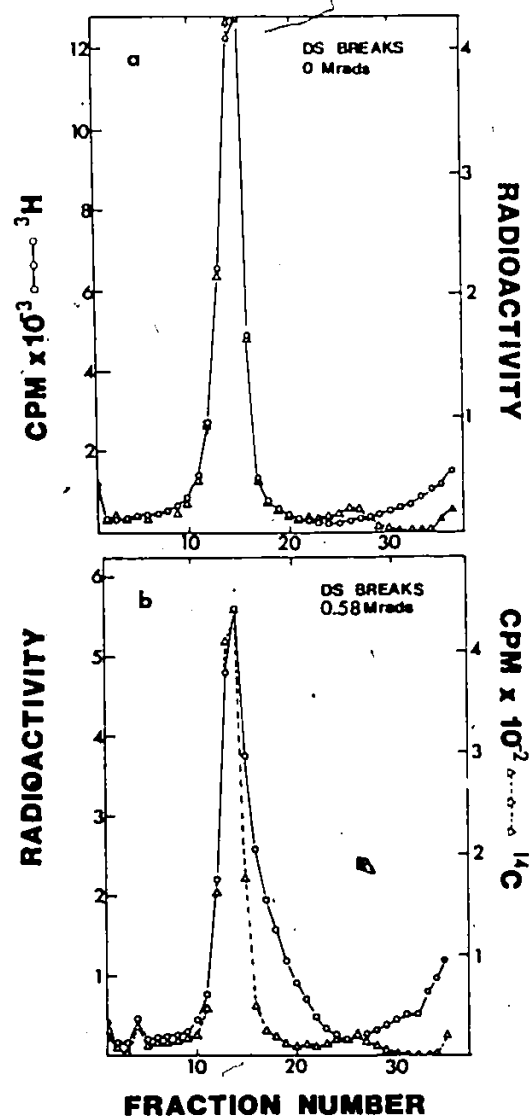
TABLE 19

DOUBLE STRAND BREAKAGE OF ADENOVIRUS 2
DNA AFTER GAMMA IRRADIATION AT 0°C

DOSE (MRADS)	FRACTION OF DOUBLE STRANDED MOLECULES UNBROKEN (SF)	AVERAGE NUMBER OF DOUBLE STRANDED BREAKS PER MOLECULE
0	0.88	0.12
0.29	0.80	0.22
0.58	0.63	0.46
0.87	0.53	0.63

Figure 17

The Average Number of Strand Breaks in Ad 2 DNA after gamma-irradiation at 0°C.

TOP PANEL (a): The average number of double strand breaks per double strand DNA molecule as a function of gamma-ray dose

BOTTOM PANEL (b): The average number of single strand breaks per single strand DNA molecule as a function of gamma-ray dose

Figure 17

$$N_o = e^{-m}$$
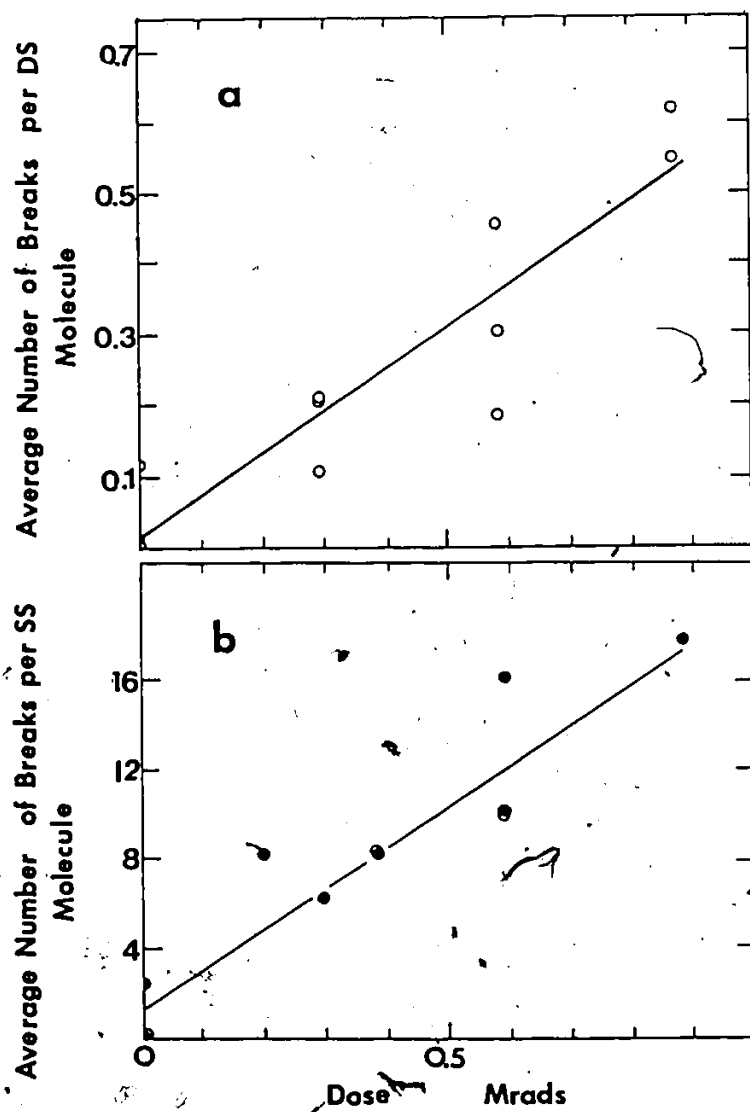
where N_o = the fraction of molecules unbroken

and m = average number of breaks per molecule can be found

in Table 19

The pooled data from three experiments has been plotted as a function of dose in Figure 17a and fitted by least-squares analysis to a straight line. The slope indicated a DNA radiosensitivity of 0.026 ± 0.004 DS breaks/rad/10^{12} daltons. The standard error was calculated using linear regression analysis.

E.II. Ad2 Single Strand Breakage after Gamma-irradiation at 0°C

In order to dissociate single-stranded DNA from protein components, irradiated virus containing ^{3}H-DNA together with marker virus containing ^{14}C-DNA was treated with NaOH and run on alkaline sucrose gradients. Radioactivity profiles of these gradients display a separation of peaks for the irradiated as compared to the unirradiated viral DNA. Typical results can be found in Figure 18. Irradiated samples showed an increase in S value (sedimentation value), indicating fragmentation of the DNA. From these profiles, the distance sedimented by unbroken single-stranded molecules (D_1) and the distance sedimented by DNA fragments from irradiated virus (D_2), were determined. From the ratio D_2/D_1, the number of breaks per molecule was determined using the relationship of Litwin et al, 1969.

Typical results for the average number of single-strand breaks per molecule are shown in Table 20 and the pooled data for three experiments are plotted against dose in Figure 17b. The average number of single-strand breaks per molecule is consistent with a linear

relationship with the slope representing a radiosensitivity of 1.7 ± 0.2 SS breaks/rad/10^{12} daltons.

E.III. Ad2 Adsorption and Plaque Formation on Human KB
Cells Following Viral Gamma-irradiation at 0°C

Irradiated and non-irradiated ^{3}H labelled Ad2 were absorbed to human KB cells at 37°C. The absorbed cells were spun down gently, washed and the radioactivity of the pallets was determined. By comparison with the counts for non-irradiated virus, the surviving fraction of the viral function of adsorption was determined. Pooled results from two experiments are shown in Figure 19a. The results are consisted with an exponential decrease of survival with a D_o of 972 ± 118 krads.

Ad2 plaques were scored on human KB cells using a neutral red, overlay method. Plaques were counted 9 days after viral infection at 37°C. Results were consistent with a D_o of 99 ± 14 krads (Figure 19a) determined from the exponential decrease of plaque formation for gamma-irradiated Ad2 upon KB cell monolayers. Comparative D_o values for loss of these viral functions following viral gamma-irradiation at -75°C were obtained by replotting the data of Rainbow and Mak, 1972 and have been shown in Table 21 and Figure 19b.

E.IV. Survival of "V" Antigen Formation for Ad2 Gamma-irradiated
at 0°C and -75°C in Normal and XP Human Fibroblast Strains

Monolayers of XP25RO and normal human fibroblast strains were infected with Ad2 which had been gamma-irradiated either at 0°C or at -75°C. Typical Vag survival curves for single experiments at 0°C or

Figure 18

Radioactivity profiles of Ad 2 DNA sedimented on alkaline sucrose gradients.

TOP PANEL (a): ^{3}H-labelled, unirradiated Ad 2
                   DNA (◯) and unirradiated ^{14}C-labelled
                   market Ad 2 DNA (△)

BOTTOM PANEL (b): ^{3}H-labelled, gamma-irradiated (0.58 Mrads)
                   Ad 2 DNA (◯) and unirradiated ^{14}C-labelled
                   marker Ad 2 DNA (△)

Figure 18
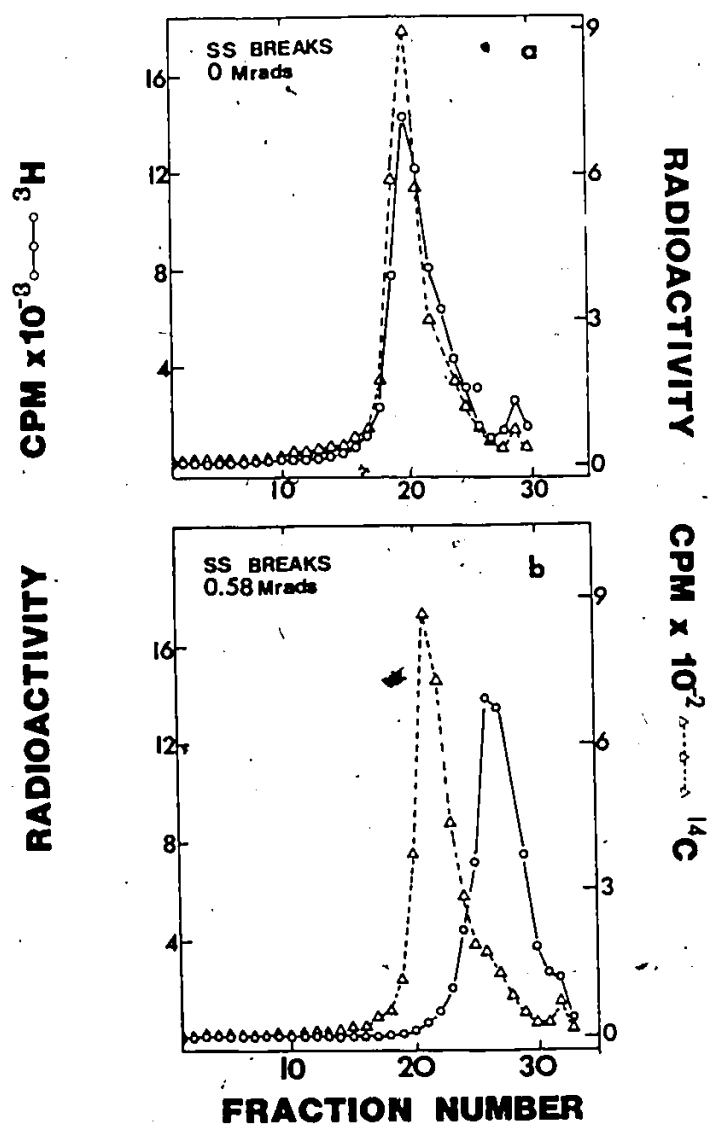
TABLE 20

SINGLE STRAND BREAKAGE OF ADENOVIRUS 2 DNA AFTER GAMMA IRRADIATION AT 0°C

DOSE (MRADS)	D_2/D_1*	AVERAGE NO. OF RADIATION INDUCED SS BREAKS PER SS MOLECULE	AVERAGE NO. OF RADIATION INDUCED SS BREAKS PER DS MOLECULE	FRACTION OF SS MOLECULES WITH NO BREAKS	FRACTION OF DS MOLECULES WITH NO BREAKS
0	1	0	0	1	1
0.29	0.589	6.35	12.70	1.7×10^{-3}	3.1×10^{-6}
0.58	0.533	10.15	20.30	3.9×10^{-5}	1.5×10^{-9}
0.87	0.452	17.80	35.60	1.9×10^{-8}	3.4×10^{-16}

* D_2 is the distance from the top of the gradient to the peak of the irradiated viral DNA.

D_1 is the distance from the top of the gradient to the peak of the marker viral DNA.

TABLE 21

COMPARISON OF BIOLOGICAL FUNCTION AND
MOLECULAR DAMAGE OF Ad2 AFTER
GAMMA IRRADIATION

	0°C	-15°C
D_0 of Virus Adsorption to KB Cells (Krads)	972±118	1936±231
D_0 of Virus Plaquing on KB Cells (Krads)	99±14	468±24
SS breaks/rad/10^{12} dalton	1.7±0.2	0.5±0.04
SS breaks/lethal hit	3.9±1.0	5.4±1.6
SS breaks/lethal hit (corrected for loss of viral adsorption)	4.4±1.7	7.9±2.9
DS breaks/rad/10^{12} dalton	0.026±0.004	0.0100±0.0025
DS breaks/lethal hit	0.059±0.017	0.078±0.016
DS breaks/lethal hit (corrected for loss of viral adsorption)	0.065±0.020	0.104±0.033
Ratio of SS breaks/DS breaks	65	50
D_0 of Vag formation of gamma irradiated Ad2 in XP25RO as a percentage of that obtained on normal strains •	88	57

Figure 19

Inactivation of Ad 2 Biological

Functions after Gamma-Irradiation

Figure a: Inactivation of viral adsorption (●) and plaque
formation (■) in human KB cells after viral
gamma-irradiation at 0°C (liquid conditions)

Figure b: Inactivation of viral adsorption (●) and plaque
formation (■) in human KB cells after viral
gamma-irradiation at -75°C (frozen)

Figure 19
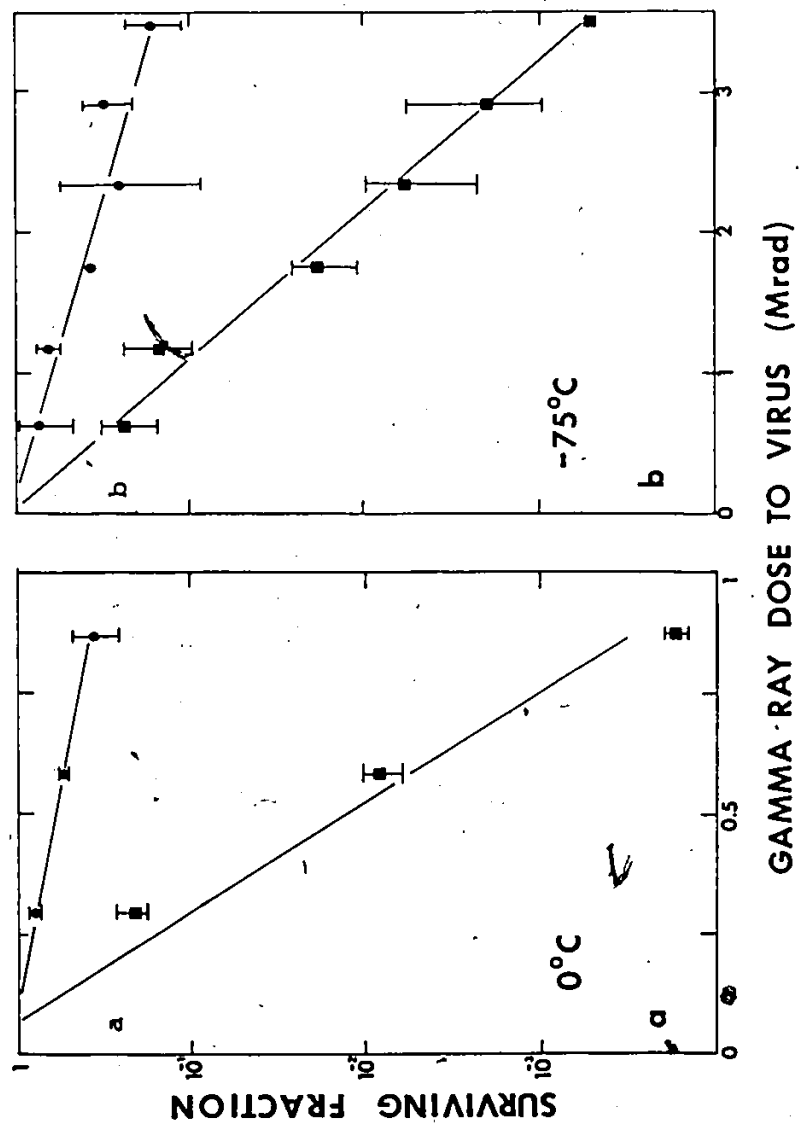
.Figure 20

Survival of Vag formation for gamma-irradiated Ad 2 in normal
and XP 25RO strains. The relative number of Vag positive cells was
determined in duplicate at three serial dilutions for each dose to the
virus. Regression analysis was used to fit the data points to straight
lines.

Panel a: Virus gamma-irradiated at 0°C (indirect conditions)

           △ CRL1221

           □ GM2674

           ● XP25RO

Panel b: Virus gamma-irradiated at -75°C (direct conditions)

           △ CRL1221

           □ GM2674

           ● XP25RO

Figure 20

SURVIVING FRACTION
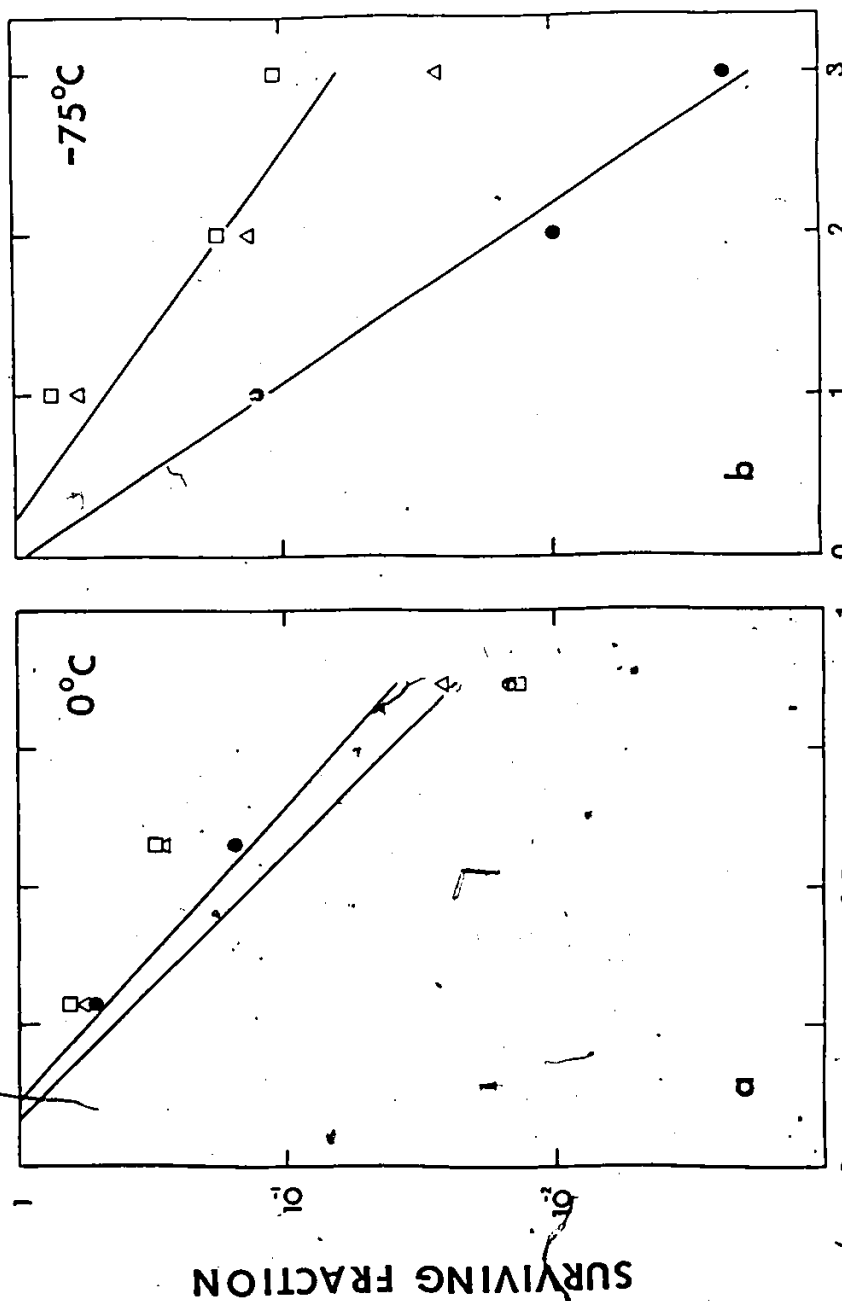
GAMMA·RAY DOSE TO VIRUS (Mrad)

TABLE 22

The D_o's* of V antigen formation for Ad2 gamma-irradiated at either 0°C or -75°C in normal human fibroblasts and in XP25RO.

CELL STRAIN	GAMMA-IRRADIATION CONDITIONS	
	-75°C	0°C
Normals:		
Hff	769	226
CRL1221	790	228
GM2803	503	238
Mean	687	231
Xeroderma Pigmentosum:		
GM710	321	198
% HCR; D_o* of Vag formation for γ-irradiated Ad in XP25RO as a % of that in normal	47	86
Mean % HCR values (range of 3 experiments)	57 (47-78)	88 (86-91)

* D_o values expressed in Krads

-75°C can be found in Figure 20 for 2 normal and XP25RO fibroblast strains. The results for a typical experiment are summarized in Table 22. Mean percentage HCR values for 3 separate experiments were determined and can be found in Table 22.

Using Ad2 gamma-irradiated at 0°C resulted in the XP fibroblast strain producing survival curves having a mean % HCR value about 88% of that using normal strains (Table 22). It can be seen that for 3 experiments the mean % HCR values for the XP strain compared to normals was lower for adenovirus irradiated at -75° (57%) compared to that for virus irradiated at 0°C (88%). A typical survival curve used to calculate the D_o can be seen in Figure 20a. Survival curves for virus gamma-irradiated at -75°C are depicted in Figure 20b indicating that infection of XP25RO fibroblasts with Ad2 gamma-irradiated at -75°C resulted in a reduced survival for Vag formation when compared to that observed for Ad2 gamma-irradiated at 0°C.

E.V.   Correlation of DNA Breakage and Biological Functions

To correlate SS and DS DNA breaks with the inactivation of biological viral functions, the fraction of the viral DNA molecules containing no radiation induced breaks (Po) was calculated from the Poisson distribution. The fraction of DS DNA molecules with no DS breaks is presented in Table 19 while the fraction of DS molecules with no SS breaks can be found in Table 20. Adsorption of the virus to the host cells was inhibited by gamma-irradiation (Figure 18) making a correction for the loss of adsorption necessary (Table 21). For the population of virus that absorbed to the host cell, an average of 4.4 ± 1.7 SS breaks and 0.065 DS breaks were induced in the viral DNA per

lethal hit after gamma-irradiation at 0°C. Values obtained previously from this laboratory after gamma-irradiation at -75°C (Rainbow and Mak, 1973) have also been included in Table 21 for comparative purposes.

DISCUSSION

I. Analysis of the Mutational System Used

The biological system used to determine the frequency of induced mutational events can influence the nature of the results obtained (Miller, 1985). Care must be taken in comparative data analysis since every type of selection system has some built in bias. Even selection pressure as broad as that requiring inactivation of a protein can yield different results for different parts of the same gene (Miller, 1985). It is clear that in bacterial systems, the frequencies of either frameshift or base substitution mutations induced by UV is a function of the size and sequence of the DNA target used and the selection system employed (Wood and Hutchison, et al, 1984; Miller, 1985). The selection system employed for the progeny assay described in this study involves the reversion (to wild type) of two ts, DNA⁻ (unable to replicate DNA at the nonpermissive temperature), adenovirus mutants following viral and/or cellular UV-irradiation. The fidelity of this system is dependent on the relative contribution of host mediated repair as opposed to replication and/or repair events determined by viral gene products.

It has been generally assumed that viral DNA replication is more autonomous (from cellular functions) as the size of the viral genome increases which allows coding for more proteins involved in DNA replication (Challberg and Kelly, 1982). Some (or all) of the viral genes involved in adenoviral DNA replication may contribute either directly or indirectly to viral DNA repair processes or alternatively,

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code for specific DNA repair functions as observed in bacteriophage T4 (Bernstein, 1981).

Adenovirus codes for 3 proteins directly involved in the replication of the viral genome and 1 protein involved indirectly in the modification (cleavage) of one of the replication proteins (Stillman, 1983). The replication enzymes include a DNA polymerase, terminal protein precursor (pTP) and a single-stranded DNA binding protein (DBP), (Challberg and Kelly, 1982). For DNA replication in vitro, two cellular factors (factor I and factor II) are required such that double-stranded DNA (Type I) intermediates and not the displaced SS DNA intermediates are replicated (Stillman, 1983). Presumably, the SS DNA which circularizes by base pairing of the terminal redundancy requires cellular factors other than cellular Factor I and II to replicate in vitro (Type II replication). Since viral DNA replication is a prerequisite of viral "V" antigen expression and viral plaque formation in human cells, the survival of these functions after viral damage can be used to detect deficiencies or abnormalities in DNA repair and/or replication in human fibroblast strains. A number of human hereditary disorders that are characterized by an increased sensitivity to DNA damaging agents also display deficiences in their ability to repair DNA damage (Rainbow, 1981; Bridges, 1981; Defais et al, 1983). Deficiencies in cellular DNA repair/replication processes as reflected using adenovirus probes may include defects in host cell expression of accessory replication proteins (factors I and II) as well as those genes specifically involved in DNA repair processes. Viral genes themselves probably play an active role in the repair and

maintenance of viral replication fidelity since damaged adenovirus or SV40 demonstrate greater decreases in HCR in XP cells as compared to damaged HSV which implies that genomes with greater coding capacity are less dependent on cellular DNA repair enzymes (Rainbow, 1981; Defais et al, 1983). HSV synthesizes its own DNA pol which plays a major role in the control of viral mutagenesis during replication and may participate in DNA repair processes (Hall and Almy, 1982; Hall et al, 1983). The recent discovery that adenovirus also codes for its own DNA pol suggests that like HSV, this enzyme may have significant control over the UV-induced mutagenesis observed among the adenovirus progeny from infected fibroblasts.

This study has examined the UV-induced mutagenesis among the adenovirus progeny resulting from infections of normal and repair deficient fibroblasts with Ad5ts36 or Ad5ts125. The induction of phenotypically wt revertants were determined among the progeny of these DNA$^-$ mutants. Revertants were therefore selected through a process which limits fidelity at the vicinity of the ts locus whereas replication/repair functions must maintain high fidelity throughout the remainder of the genome to allow the biological function of plaque-forming ability (progeny production) to survive. A random mutagenesis throughout the "late" (structural proteins) portion of the viral genome would be expected to severely limit the ability of damaged virus to produce viable viral progeny. The ability of this system to detect viral error-prone repair/replication events requires a selective hypermutability at or near the ts locus to produce a revertant with wt growth properties while genomic DNA fidelity is maintained at a level

sufficient to maintain plaquing ability. This suggests that for the progeny assay to indicate error-prone processes, "fidelity" of repair/replication must be selectively reduced at the ts locus in a sequence specific "targeting" mechanism. Alternatively, error-free (hypomutable) events should be easily detectable since increased fidelity of repair/replication processes would not be expected to interfere with plaque forming ability of progeny. Increased viable progeny production may therefore indicate the occurrence of error-free replication "events".

Sequence data obtained from targeted mutational events among SV40 ts mutant revertants induced by UV-light have indicated that the original ts mutation was still present in all revertants analyzed (Bourre and Sarasin, 1983). The revertants were all second site substitution mutations able to suppress the ts nature of the tsA58 mutation of SV40. They were located in the C terminal region of the protein (over a range of about 10^3 bp) and occurred opposite TT or TC sequences (Bourre and Sarasin, 1983). Revertants were selected by plaquing UV-irradiated SV40 tsA58 on control or preirradiated CV1-P monkey kidney cells at the nonpermissive temperature (41°C) at a high dilution (Bourre and Sarasin, 1983). In this study, 20 Ad5ts36 progeny plaques were similarly picked from HeLa indicator monolayers at 39°C (nonpermissive) after the virus had been UV-irradiated and grown in either control or UV-irradiated normal fibroblast monolayers (data not shown). Limiting dilutions of the plaque picks were plaqued at 33° and 39°C on HeLa monolayers to determine if virus scored at 39° was representative of "true" revertants. All 20 samples produced similar

plaque titres at 33°C compared to 39°C which suggested that the plaques originally scored at 39° were phenotypically wt. As with SV40, it is possible that Ad5 reversion at the ts loci involves second site reversion able to suppress the mutant phenotype. The high degree of UV-induced reversion of Ad5ts36 and Ad5ts125 strongly suggests that these ts mutations are point (substitution) mutations. Sequence data available for Ad5ts125 confirms that the ts phenotype is the result of a point mutation in the 72K DNA binding protein (Kruijer et al, 1982).

The comparison of error-prone Ad5 repair/replication processes in normal and repair deficient fibroblasts must be made with the realization that the final outcome is the result of a complex interplay of constitutive and/or inducible error-prone/error-free processes mediated by a combination of host and viral gene products. Observable differences in the "RF" for adenoviral progeny from normal and repair deficient fibroblasts depends on the repair contributions of these error-free and error-prone modes of repair. Lethality is representative of DNA lesions that escape both modes of repair while mutagenesis results from lesions that escape error-free repair but are processed by an error-prone mechanism. The relative expression of UVEM upon comparing normal and repair deficient fibroblasts, may indicate differences in host gene products regulating mutagenic responses to DNA damage. Similarly, a study of the kinetics of UV-induced viral mutation frequencies may help in proposing molecular models of UV-mutagenesis in normal and repair deficient human fibroblasts.

II. The Growth Kinetics of Progeny Resulting from Infections of

Unirradiated or UV-irradiated Normal Human Fibroblasts with

Unirradiated and UV-irradiated Ad5ts36

a) UVER Values Associated With Viral Infections

Immediately Following Cellular UV-irradiation

The comparison of Ad5 UVER and UVEM data obtained in this study

to viral enhanced reactivation and enhanced mutagenesis data from other

studies must be made with the realization that many factors contribute

to the expression of these parameters of DNA replication and/or repair.

Such factors include the contribution of viral or cellular functions

dictated by the choice of host cell and viral probe used, the viral

function assayed, the nature of the viral DNA damage, the time delay of

viral infection following cellular induction, method of induction (ie

direct vs indirect), the multiplicity of viral infection, as well as

the state of the cells in terms of confluency, passage number and

cultural conditions. Differences in the UVER/UVEM responses observed

in comparing separate viral studies may be attributable to any one or

more of the above factors.

In this study, an indirect method (progeny production) was used

to characterize the UVER responses observed for Ad5ts mutants grown

through a single lytic cycle in human fibroblasts. In order to examine

UVER/UVEM responses, it was necessary to construct a single cycle lytic

(permissive) growth curve of Ad5ts36 and determine the maximal growth

time before multiple rounds of replication occurred. This was done in

control and UV-irradiated normal human fibrobasts (Hff) with virus that

was unirradiated or UV-irradiated (Figure 1) and infections were

immediately following cellular UV-irradiation. Similar growth curve
experiments have been described previously for parvovirus H-1 (Cornelis
et al, 1982), SV40 (Sarasin and Benoit, 1980) and HSV (Coppey and
Menezes, 1981) but not for adenovirus; however, UV-irradiation of Ad2
has been reported to delay the onset of DNA synthesis and subsequent
progeny production in unirradiated KB cells (Mak and Mak, 1974). Among
the viral probes, only HSV has also been examined for growth kinetics
when viral infections were immediately following cellular
UV-irradiation. HSV growth was delayed (10 hours) after UV-irradiation
of the virus (300 J/m^2) in both unirradiated and UV-irradiated human
fibroblasts (Coppey and Menezes, 1981). Also similar to the data
obtained in this study (Figure 1) was the reduced production of progeny
from UV-irradiated HSV infecting UV-irradiated cells as compared to
unirradiated fibroblasts. Although the lytic cycle of HSV is faster
than that observed for Ad5, the viral yields and time delays observed
for the appearance of exponential growth for UV-irradiated virus were
very similar between the two viruses in normal human fibroblasts. With
HSV, the growth of intact virus was hardly impaired in UV-irradiated
cells compared to control monolayers while growth of UV-irradiated HSV
in UV-irradiated cells was severely impaired resulting in reactivation
factors less than one and similar to that observed for Ad5 (Figure 1).
The decreased UVER for both Ad5 and HSV (immediate infections) was the
result of a delay in the onset of exponential growth of progeny from
UV-damaged virus infecting UV-irradiated cells as well as a decrease in
the yields observed under these conditions (when compared to the
progeny growth of UV-irradiated virus infecting unirradiated

fibroblasts). Under conditions where virus was infected immediately following cellular induction, Ad5 displayed UVER's ranging from 0.10 - 0.79 in Hff fibroblasts (Figure 1). Normal strains infected immediately after cellular UV exposure with intact and UV-irradiated HSV displayed UVER's that ranged from 0.05 - 0.2 (Coppey and Menezes, 1981). The lower UVER values for UV-irradiated HSV (300 J/m^2 Coppey and Menezes, 1981) as compared to Ad5 (1200 J/m^2) was observed at similar progeny surviving fractions in normal fibroblasts of 4×10^{-3} and 3×10^{-3} respectively. The lower UVER values observed for UV-irradiated HSV may be dependent on the shorter HSV lytic cycle when compared to that of adenovirus. Alternatively, a higher UV-dose to cells (15 J/m^2) used for the HSV UVER studies as compared to that used in this study (10 J/m^2, immediate infections Figure 1) may have contributed to the lower UVER factors observed for HSV. These results suggest that viral enhanced reactivation was deficient in normal fibroblasts when either Ad5 or HSV progeny production was assayed using an immediate infection protocol. It is apparent that UV-irradiation of either virus blocks the onset of viral progeny production and that UV-irradiation of the host lengthened the blockage period.

b) UVER Values Associated With Viral Infections Delayed
   24 Hours Following Cellular UV-irradiation

To examine the inducibility of UVER responses, growth curves were repeated such that viral infections were delayed 24 hours post

cellular UV-irradiation. The major effect of this delay was observed in the growth curves of UV-irradiated virus (Figure 1). The yield of progeny from UV-irradiated Ad5 was increased by preirradiation of the fibroblasts when compared to that for UV-irradiated virus in unirradiated fibroblasts. This was very similar to the increased progeny production of UV-irradiated HSV infecting fibroblasts that were preirradiated 36 hours before viral infection (Coppey and Menezes, 1981). The progeny production from intact Ad5 (this study) or HSV virus (Coppey and Menezes, 1981) was very similar in control or UV-irradiated fibroblasts and UVER values that were greater than one were observed for both viral systems. The maximal UVER of UV-irradiated HSV occurred when viral infections were delayed 36 hours after UV-irradiation of normal fibroblasts (Coppey and Menezes, 1981) whereas the maximal UVER for UV-irradiated Ad5 occurred when viral infections were delayed 24 hours after UV-irradiation of normal fibroblasts (Figure 5, this study). The difference observed in the infection delay periods (24 vs. 36 hours) that elicit the maximal UVER of UV-irradiated Ad5 and HSV in normal human fibroblasts may rely in part on the increased length of the lytic cycle of Ad5 as compared to HSV. Furthermore, Ad5 progeny assays were conducted at 33°C (this study) as compared to 37°C for HSV (Coppey and Menzes, 1981) and this would further extend the Ad5 lytic cycle as compared to HSV in normal fibroblasts. These results suggest that some transient time course dependent viral function involved in progeny production (possibly viral DNA replication) may the "target" sensitive to the UVER phenomenon of normal fibroblasts.

A similar growth curve (24 hour delay) was constructed for intact or UV-irradiated SV40 in control of UV-irradiated (10 J/m^2) monkey kidney cells (CV-1P) at 33°C (Sarasin and Benoit, 1980). As with Ad5 and HSV, the production of progeny from UV-irradiated SV40 was delayed and inhibited when compared to progeny production of intact virus infecting either UV-irradiated or unirradiated cells. These results using three different viral probes suggest that UV-inducible cellular repair functions enable UV-irradiated virus to produce progeny earlier and to a greater magnitude than in unirradiated cells. This enhanced reactivation demonstrated a damage specific component since unirradiated virus was not reactivated to the same degree following preirradiated preirradiation of cells.

Single-cycle growth curves have been similarly constructed for unirradiated and UV-irradiated parvovirus H-1 (Hamster-osteotylic virus) grown in preirradiated (14 hour delay) or unirradiated NB-E cells (Cornelis et al, 1982). Preirradiation of the host monolayers (4.5 J/m^2) increased the progeny yield specifically for UV-irradiated virus and not for intact H-1 virus. This resulted in progeny enhanced reactivation as observed in this study and those using SV40 (Sarasin and Benoit, 1980) and HSV (Coppey and Menezes, 1981). Thus, the phenomena of UVER can be observed using a variety of viral probes in different cell types using progeny assay techniques. Since the viral probes each utilize different replicative strategies, the generalized phenomenon of enhanced viral reactivation may be representative of a repair process active before replication takes place or occurs during a very fundamental step of DNA replication. An inducible mutagenic

repair function that "modified" viral replication in a damage independent manner would be expected to equally reduce the delay observed for progeny production from intact or UV'D virus infecting cells UV-irradiated immediately after infection. Since this was not the case, the data suggests that UVER is representative of a UV damage dependent (targeted) repair function able to tolerate or overcome potentially lethal lesions that would normally block replication in uninduced cells.

The assays described above, all describe UVER in terms of viable progeny released after single cycle growth in permissive host cells. Virus survival as measured by plaque formation is an endpoint further removed from the initial damage given to cells or virus and the "target" of UVER in the steps leading to plaque formation is not known. It was therefore considered of interest to examine the Ad5ts36 lytic cycle and viral UVER using as an assay, an event closer (in time) to viral replication. An assay using "V" ag expression as an endpoint was therefore used to determine UVER (Figure 2) and conducted under similar experimental conditions to the assay described by Figure 1 (D,E,F). The time course of "V" ag expression of unirradiated and UV-irradiated Ad5ts36 in control and UV-irradiated host cell monolayers (grown at 33°C) can be compared to similar curves generated by infections of unirradiated and UV-irradiated Ad2 into control or UV-irradiated normal fibroblasts (immediate infection following cellular induction, Jeeves and Rainbow, 1983a). Both studies produced UVER factors greater than one over the entire time course as the result of the increased expression of UV-irradiated virus in UV-irradiated cells as compared to

unirradiated cells, while intact viral "V" ag expression was inhibited by preirradiation of the host monolayers. UV-irradiation of cells resulted in, not only an increased amount of Vag expression, but also in an earlier onset of Vag expression in cells infected with UV damaged virus. UVER factors for UV-irradiated adenovirus produced using the adenovirus "V" ag assay were similar to those expressed for adenovirus progeny production (Figures 1 and 2). The average UVER of progeny (Figure 1F) was 3.0 $\pm$ 1.3 while in Figure 2 (Vag) the average UVER was 5.1 $\pm$ 1.0 ($\pm$ 1SE) using Ad5ts36 as a probe of Hff cells that were infected 24 hours following cellular irradiation.

Maximal UV enhanced reactivation factors have been determined for a number of cell types using a variety of viral probes. UV-irradiated CV-1 monkey cells were found to express a maximal ER of ca. 32 for UV-irradiated SV40 using a progeny assay as an end point (Sarasin and Benoit, 1980). Sarasin and Hanawalt (1978) reported a maximal UVER of ca. 7 whereas Bockstahler and Lytle (1977) reported a maximal UVER of ca. 2 for UV-irradiated SV40 in CV-1 cells.

A maximal mean UVER for UV-irradiated adenovirus of 3.1 was detected in pooled normal human fibroblasts using a V antigen assay as an end point (Jeeves and Rainbow, 1983). Normal levels of UVER (2-4) were detected in 2 CS, 2XPA and 2XPD human fibroblast strains for UV-irradiated Ad2 (Jeeves and Rainbow, 1983b) XP variant strains were reduced (as compared to normals) in the expression of maximal UVER for UV-irradiated Ad2 (Jeeves and Rainbow, 1983b). Maximal UVER values of 2.0 were found for UV-irradiated HSV in CV-1 (Bockstahler and Lytle, 1970) or human KD cells (Lytle et al, 1976) and XP fibroblasts produced

maximal UVER values of 3 for UV-irradiated HSV (Lytle et al, 1976). A
maximal UVER (of viral progeny) for UV-irradiated HSV-1 in CV-1 of ca.4
have been described for CV-1 (Lytle et al, 1980) and human NB-E cells
(Lytle and Knott, 1982). Maximal UVER values of 1.8, 3.0, 2.2, 2.0 and
2.7 (Table 1) have been described for UV-irradiated HSV in normal, XPA,
XPC, XPD and XP variant human cells (Abrahams et al, 1984). Maximal
UVER values of ca. 2 were described by Cornelis et al (1982) for
UV-irradiated parvovirus H-1 in determined in human cells. The above
UVER values determined in human cells compare favorably to the maximal
mean UVER values for UV-irradiated Ad5 (this study) 3.4 (Table 5), 3.0
(Figure 1) and 5.1 (Figure 2) obtained in normal human fibroblasts. It
also appears that HSV may express lower UVER values in human cells when
compared to those obtained using adenovirus as a probe.

   In general, the assays described by Figures 1 and 2 are
illustrative of the generalized phenomenon of induced viral
reactivation where treatment of mammalian cells with physical or
chemical DNA damaging agents prior to infection with UV-irradiated
virus, enhances viral survival. This phenomenon has been found for a
variety of replicating virus, in a number of different host cells,
using inducing agents which are able to interrupt cellular DNA
synthesis. Gamma-irradiation of normal human fibroblasts leads to the
enhanced reactivation of both gamma and UV-irradiated Ad2 (Jeeves and
Rainbow, 1979a, b). Since gamma and UV-irradiation each produce a
different spectrum of induced DNA lesions, it suggests that ER may
involve 2 separate repair modes or alternatively a single broad
spectrum repair mode (Rainbow, 1981). The exact nature of ER is

unknown, however for HSV multiplicity reactivation does not appear to be involved (Lytle, 1978) and it occurs independent of cellular excision repair (Lytle et al, 1976a, b).

ER phenomena display an "inducible" nature with reactivation factors peaking when viral infections were delayed following cellular induction (Lytle et al, 1978; Sarasin and Hanawalt, 1978; Lytle and Goddard, 1979, Table 1). The "inducible" nature of UVER in this work was evident by comparing Figure 1(A,B,C) and 1(D,E,F,). Other workers have found this "inducible" viral recovery system requires de novo protein synthesis since the inhibitor cyclohexamide blocks the expression of UVER when it is present immediately following cellular irradiation (Das Gupta and Summers, 1978; Lytle and Goddard, 1979; Rommelaere et al, 1981; Vos et al, 1981; Cornelis et al, 1981; Su et al, 1981).

Agents able to interrupt DNA synthesis will also induce enhanced viral reactivition in mammalian cells (Fogel et al, 1979; Lytle and Goddard, 1979). This suggests that inhibition of DNA replication may "signal" the induction of a repair response that results in the expression of viral ER phenomena. Phage ER phenomena have been correlated with the induction in E. coli. of the "SOS" regulon which expresses DNA repair functions following cellular DNA damage (Little and Mount, 1983; Walker, 1984). Phage ER phenomena which are accompanied by enhanced phage mutagenesis are thought to reflect the induction of this inducible "error-prone" DNA repair mode which is also capable of enhancing bacterial chromosomal mutagenesis (Walker, 1984). DNA damage can also mediate the induction of prophage

In lysogenic bacterial hosts (Defais et al, 1983; Little and Mount, 1983; Walker, 1984). A mammalian equivalent to prophage induction has been described for the induction (activition) of latent virus from SV40 transformed mammalian cells (Kaplan et al, 1975). Similarly, UV-irradiation of AGMK cells induced an activity that stimulated SV40 rescue from transformed Syrian hamster cells after cell fusion with induced AGMK cells (Nomura and Oishi, 1984). This SV40 induction was quite similar to the indirect activation of bacteriophage lambda requiring the RecA protease function which is an integral regulatory part of the SOS regulon (Little, 1983).

DNA lesions (such as pyrimidine dimers) completely block eukaryotic DNA polymerases in viro or in vitro (Hall and Mount, 1981). When the replication of UV-damaged templates is blocked at a lesion, new chains may be initiated beyond the dimer leaving a gap (Lehman, 1972; Hall and Mount, 1981). In SV40 virus replication, it has been suggested that the gaps are filled by an error-prone DNA synthesis or through a strand insertion mechanism which occurs only on retrograde strand synthesis (Sarasin and Hanawalt, 1980) or alternatively on either strand (Edenberg, 1983).

Recent studies with SV40 replication have suggested that at UV damaged sites, replication was not blocked but proceeded rapidly past dimers creating a gap which was subsequently slowly filled (White and Dixon, 1984). Transdimer synthesis by an inducible "error-prone" DNA polymerase may in the case of SV40 replication, be responsible for targeted reversion events.

The existance of a mammalian equivalent of the bacterial SOS

response would be implied if it could be established that there existed an inducible enhanced mutagenesis concomitant with ER of damaged viral probes. Existence of such a mammalian error-prone repair mode would therefore suggest a possible mechanism in the activation of viral and cellular oncogenes that play a central role in cellular transformation and oncogenesis.

III. a. UV-Enhanced Reactivation and Mutagenesis of UV-irradiated Adenovirus in Normal Human Fibroblasts

Normal human fibroblasts were probed for error-prone replication/repair events using the adenovirus DNA$^-$ mutants Ad5ts36 and Ad5ts125. Fibroblasts were infected at either 0, 12, 24, 36 or 48 hours following cellular induction in order to examine the "inducible" nature of the various parameters of UV-mutagenesis (Figure 5). This data was compared to the UV-induced reversion of Ad5ts2 progeny in normal human fibroblast strain CRL1187 (Day and Ziolkowski, 1981). In the latter study cells were induced with a UV dose of 5 J/m^2, 24 hours prior to viral infection with unirradiated or UV-irradiated Ad5ts2. An increase in the UV-induced reversion frequency for preirradiated normal fibroblasts was not observed at viral UV doses of 200, 400 or 600 J/m^2. There was a small (but not significant) increase in UV-induced reversion (UV-irradiated cells) at a dose of 800 J/m^2 to the virus (Day and Ziolkowski, 1981). UVER at all these doses was slightly greater than one (from Figure 1; Day and Ziolkowski, 1981). From these data it was concluded that the production of phenotypic wt revertants was not significantly affected by preirradiation of the host monolayers. Data

presented in this study suggests that there is a small UVEM (1.4 ± 0.2) concomitant with UVER (3.4 ± 0.8) when UV-irradiated virus was infected 24 hours after cellular irradiation (Table 5). For these experiments (Table 5) the average number of lethal hits was 8.1 ± 0.6 (Table 5), corresponding to a viral progeny survival considerably smaller than that of Ad5ts2 after a UV dose of 800 J/m^2 as described by Day and Ziolkowski (1981). In another study measuring Ad2ts1 survival and mutagenesis in HeLa cells, virus was irradiated at 200, 400, 600 and 800 J/m^2 which resulted in a viral survival of about 7×10^{-3} for virus UV-irradiated at 800 J/m^2 (Piperakis and McLennan, 1984b). This survival was also higher than the pooled survival observed in Table 5. The inability to detect UVEM in the previous two studies (Day and Ziolkowski 1981; Piperakis and McLennan, 1984) may therefore be the result of the reduced lethal damage induced by lower viral UV-doses as compared to those observed in Table 5. UV-irradiation of virus in these latter studies resulted in progeny lethal hit values less than 6. This is below the damage level needed to detect UVEM as illustrated by Figure 9. HeLa cells (as with normal fibroblasts; Day and Ziolkowski, 1981) produced Ad5ts2 UVEM values that were approximately 1.0 at UV doses of 200, 400 and 600 J/m^2 to the virus (Piperakis and McLennan, 1984) while at a dose of 800 J/m^2 to the virus, the UVEM was less than one. This indicated a lack of UV enhanced mutagenesis among the reactivated virus obtained from preirradiated HeLa monolayers (Piperakis and McLennan, 1984). Intact virus produced an untargeted increase greater than one when infections were delayed 18 hours following cellular UV-irradiation (Piperakis and McLennan, 1984)

similar to the untargeted increase 1.6 $\pm$ 0.3 observed for adenovirus in pooled normals when infection was delayed 24 hours following cellular induction (Figure 5).

All the above studies have indicated a UV dose dependent increase (targeted) for viral reversion in unirradiated (control) fibroblasts. The kinetics of the UV dose dependent increase in a number of viral reversion systems have suggested that the magnitude of targeted mutagenesis increased as a square of the dose to virus (Cleaver and Weil, 1975; Day and Ziolkowski, 1981; Sarasin et al, 1981). A UV-dose dependent increase in RF of UV-irradiated SV40 was observed in unirradiated CV-1 (monkey) cells (Cleaver and Weil, 1975). A plot of log RF versus log UV fluence in the latter study resulted in a linear relationship with a slope of 2. This agrees with the data of Sarasin et al. (1981) which found a slope of 2.4 for the curve obtained from a log-log plot of UV-induced RF versus log UV-dose for SV40 in unirradiated CV1-P cells. Similarly, a UV-dose dependent increase in RF of UV-irradiated Ad2 was observed in normal human fibroblasts (Day and Ziolkowski, 1981). The slope of the curve given by the log UV-induced RF versus log fluence to UV-irradiated Ad2 was 1.9 (for normal human fibroblasts) or 2.1 (for A498 a human kidney carcinoma cell line). The significance of "2-hit" kinetics to the molecular mechanism of UV-induced viral mutagenesis has been speculated on for some time (Witkin, 1976) and will be discussed in a subsequent section.

III.b. <u>The Separate Regulation of Untargeted and Targeted Increases</u>

<u>Induced by UV-irradiation of Normal Human Fibroblasts</u>

The targeted increase values in normal human fibroblasts increased until 24 hours into the time course followed by decreases at later times (Figure 6), whereas the untargeted increase was maximally expressed when viral infections were immediately after or delayed 12 hours following cellular UV-irradiation. In contrast, an "inducibility" for both targeted and untargeted increases was observed for UV-irradiated HSV-1 in UV-irradiated Vero monolayers (Das Gupta and Summers, 1978). Untargeted and targeted increases greater than one were observed when virus was infected 6 hours after cellular irradiation but not when infection was immediately following cellular induction (Das Gupta and Summers, 1978). An "inducible" untargeted increase has also been described for SV40 produced in BSC-1 monolayers (Cornelis et al, 1981b) or for HSV in normal and repair deficient human fibroblasts (Abrahams et al, 1984). In the latter study, maximal untargeted increases and UVER were obtained at similar infection delay periods (24 or 48 hours after cellular UV) for UV-irradiated HSV in normal human fibroblasts. Similarly, XP group A, C and D fibroblasts displayed a maximal untargeted increase when viral infections were delayed 24 or 48 hours after cellular UV which was concomitant with the maximal UVER of UV-irradiated HSV (Abrahams et al, 1984). In XP variant fibroblasts the maximal untargeted increase was observed when infection was delayed 72 hours after cellular UV, whereas maximal UVER occurred when viral infection was delayed 24 hours following cellular irradiation (Abrahams et al, 1984). This led to the suggestion that

separate mechanisms were responsible for UVER of UV-irradiated HSV and EM (untargeted) of intact HSV (Abrahams et al, 1984). The concomitant time course dependent expression of the maximal untargeted increase and maximal ER have also been reported for UV-irradiated H-1 (Cornelis et al, 1981), SV40 (Cornelis et al, 1980) and HSV-1 (Das Gupta and Summers, 1978). Since these maxima all occur at delayed times following cellular treatment it is tempting to speculate that they represent inducible phenomena. Furthermore, the protein synthesis inhibitor cyclohexamide has been shown to inhibit enhanced mutagenesis and reactivation of UV-irradiated H-1 (Cornelis et al, 1981; Su et al, 1981) and HSV-1 (Das Gupta and Summers, 1978) indicating that these "inducible" phenomena may depend on de novo protein synthesis. In this study, maximal untargeted increases were not concomitant with the maximal UVER obtained for UV-irradiated Ad5 in normal human fibroblasts (Figure 5). This may be due, at least in part, to differences in the length of the lytic cycle and/or the replicative strategy of adenovirus when compared to that of parvovirus H-1 (Cornelis et al, 1982), SV40 (Sarasin and Benoit, 1980) and HSV (Coppey and Menezes, 1981).

The delayed expression of the maximal targeted response as compared to the immediate expression of the maximal untargeted response (Figure 6) suggested an independent regulation of these two processes in normal human fibroblasts. Log-log plots of either untargeted increase or targeted increase vs UVEM for human fibroblast strains (data not shown) demonstrated that the untargeted increase was negatively correlated with UVEM while targeted increase was positively correlated with UVEM. The correlations were evident in both normal and

repair deficient fibroblasts and suggests that repair functions responsible for the untargeted and targeted increases were not covariant but separately regulated. The delayed nature of the appearance of maximal targeted increases compared to the immediate expression of maximal untargeted increases, also strongly implies that the molecular mechanism of untargeted mutagenesis is different from targeted UV-mutagenesis.

It has been similarly proposed for lambda UV mutagenesis in E. coli) (Wood and Hutchinson, 1984) that the mechanism of mutagenesis of undamaged phage lambda (in irradiated host) is different from the mechanism of mutagenesis of damaged phage. The separate genetic requirements of indirect (untargeted) and direct (targeted) UV mutagenesis also suggests these processes are different (Maenhaut-Michel and Caillet-Fauquet, 1984). UV-induced mutagenesis of phage lambda may occur by two recA-dependent mechanisms: targeted UV mutagenesis that requires umuC and recF and untargeted mutagenesis which is independent of umuC but requires uvrA and polA gene products (Maenhaut-Michel and Caillet-Fauquet, 1984).

III.c. Possible Molecular Mechanisms of the Untargeted Response

A possible candidate for the decreased fidelity of intact viral replication in UV-irradiated normal cells may be deoxyribonucleoside triphosphate (dNTP) pool imbalances (Kunz, 1982). UV-irradiation of the CHO line V79 caused rapid increases in cellular dNTP concentrations characteristic of imbalance of dNTP pools at the DNA replication fork (Das et al, 1983). In a similar study, CHO cells that were

UV-irradiated displayed induced changes in dNTP concentrations within the first 10 minutes following UV exposure (Newman and Miller, 1983). The depletion of thymine nucleotides is mutagenic in prokaryotes and induces point mutations in the DNA of eukaryotic organelles while excess of thymidine or thymidylate is mutagenic in both prokaryotes and eukaryotes (Kunz, 1982). In CHO cells, a rapid increase in dTTP was observed following UV-irradiation (Das et al, 1983; Newman and Miller, 1983) suggesting that such pool imbalance may similarly facilitate adenovirus replication errors. Other factors such as nucleotide sequence specificity, mismatch repair and feedback inhibition of dNTP's on nucleotide metabolizing enzymes may also mediate reduction in fidelity of replication (Kunz, 1982).

Relaxation in fidelity of intact viral replication has been observed in many different viral systems. Parvovirus H-1, SV40, adenovirus and HSV-1 all exhibited a reduction in replication fidelity of intact, virus grown in UV-irradiated host cells (see Table 1). Cornelis et al, 1981 have described the largest untargeted increase (84 fold) observed for intact SV40 infecting BSC-1 cells 72 hours following cellular UV-irradiation. HSV-1 appears to be a poor viral indicator of untargeted increases in established cell lines since little or no decrease in fidelity was observed for intact virus grown in UV-irradiated CV-1 or NB-E monolayers (3 of 4 experiments, see Table 1). This does not seem to be the case for the non-immortal normal or XP human fibroblast cell strains which exhibit HSV-1 untargeted increases of 2-3 (Abrahams et al, 1984). The large untargeted increases associated with the latter study may be related to the

technique used to assay $TK^+ \rightarrow TK^-$ mutations. Selection for TK^- mutants occurred at 8 hours post infection such that only infectious centers producing pure TK^- clones would survive (Abrahams et al, 1984). Other studies have utilized progeny assays that determine TK^+/TK^- ratios in indicator cells where selection occurred immediately after infection (Das Gupta and Summers, 1978; Lytle et al, 1980; Lytle and Knott, 1982; Takimoto, 1983). Therefore the untargeted increases in the latter studies may be masked through the use of a progeny assay technique.

An important consideration to the discussion of the untargeted increase was the observation that exogenously damaged DNA (lethally UV-irradiated HSV) could stimulate a "trans-acting" untargeted increase in the reversion of Ad5ts36 infected 24 hours after cellular induction (adsorption of damaged HSV-1). This was clearly demonstrated for normal fibroblast strain GM2674 and XP25RO complimentation group A (Table 17). These experiments did not eliminate the possibility that HSV coded gene products may have contributed to the process; however, the UV dose to virus (HSV) prevented plaque formation on unirradiated host monolayers. The presence of lethally UV-irradiated HSV may have "signaled" the untargeted increase in a manner analagous to the induction signal hypothesized for the SOS response observed in E.coli. (Little, 1983). Such a transacting induction of a cellular "mutator" function has recently been described using UV-irradiated SV40, ϕX174 or calf thymus DNA to induce a SV40 transformed newborn human kidney cell line or a Harvey Sarcoma virus transformed rat liver cell line (Cornelis et al, 1982; Dinsart et al, 1984). A maximal untargeted increase of 3.0 was observed for intact parvovirus H-1 in cells

transfected with UV-irradiated DNA as compared to control cells (Dinsart et al, 1984). This was very similar to the untargeted increase observed in this study (3.6 ± 0.3) using lethally UV-irradiated HSV-1 to induce normal fibroblast strain GM2674 (Table 17). Furthermore, an untargeted increase of intact H1 occurred after transfection of rat cells with UV-damaged SS bacteriophage DNA or DS SV40 DNA lacking a functional origin of replication (Dinsart et al, 1984) and presumably neither DNA would be able to replicate in non-permissive rat cells. In this study it was also unlikely that lethally UV-irradiated HSV-1 replicated since at the UV dose used the UV-damage prevented plaque formation in permissive cells. Taken together these results suggest that either non replicating SS or DS UV-damaged DNA is capable of eliciting an untargeted increase.

Mating experiments using excision deficient S. cerevisiae found that up to 40% of cyc1-91 revertants induced by UV are untargeted and appear not to be induced by any diffusible factor capable of inhibiting fidelity (Lawrence and Christensen, 1982). This conclusion is surprising since in mammalian cells, exogenously added UV-irradiated DNA stimulated the untargeted increase in a transacting mechanism (this study, Dinsart et al, 1984). The untargeted mutator activity may result from a loss of replication fidelity due to sequestering of "fidelity" factors (Lawrence and Christensen, 1982) or dNTP pool imbalances. In yeast the reduction of fidelity is a nuclear limited phenomenon that depends on constitutive conditions (Lawrence and Christensen, 1982) similar to the "immediate" expression of the adenovirus untargeted increase. A "limited fidelity" model has been

proposed stating that untargeted mutations occur when the limited capacity of certain mechanisms normally responsible for fidelity are exceeded and these mechanisms are required for replication on both irradiated and unirradiated templates (Lawrence and Christensen, 1982).

III.d. Molecular Mechanisms of the Targeted Response

Examination of the molecular spectrum of UV induced mutations in E.coli (Miller, 1982; 1985) and in mammalian systems (Bourre and Sarasin, 1983: Braggaar et al, 1985) indicates that most of the UV-induced mutations are targeted to pyrimidine doublets. In mammalian cells the molecular mechanism is not clear; however, in E.coli it has been suggested that mutations induced at a premutational site are the result of a 2-step process requiring the direct participation of the activated recA gene product at the site with subsequent bypass mediated by the umuDC products (Bridges, 1985). Subsequent bypass involving the umuDC products may introduce replicative errors, peripheral to the targeted damage occurring at the premutational lesion and have therefore been termed "hitch-hiking" errors (Bridges, 1983;1985).

For yeast, in the absence of evidence for the existence of an inducible factor that transiently reduces fidelity (to allow trans-lesion synthesis) it has been proposed that yeast targeted mutations result from the limited capacity of pyrimidine dimers to form Watson-Crick base pairs. The inhibition of chain elongation results from steric hinderance of the template rather than the ability of the lesion to be instructive and when a translesion synthesis finally proceeds, mutations arise by misreplication (Lawrence and Christensen,

1982). A misreplication mechanism as described above that does not require an inducible factor for UV targeted mutagenesis is difficult to interpret in light of the observation that "2-hits" were required for the induction of targeted reversion. The two hit phenomena of UV-targeted mutagenesis may be the result of an induction or indexing signal and a premutational photoproduct that targets the mutation event (Fix and Bockrath, 1983). It has been suggested that the indexing signal may be mediated by SS DNA (exposed by excison repair) or by replicative blockage at pyrimidine dimers resulting in polymerase "idling" (Witkin, 1976; Schroeder, 1979; Little, 1983). In E.coli. the premutational photoproduct may be the 6-4 photoproduct and not thymidine dimers (Brash and Haseltine, 1982; Haseltine, 1983).

The cellular response to infections with UV-damaged viral probes include the repair of potentially lethal viral damage by cellular error-free repair (EFR) and error-prone repair (EPR) such that host cell reactivation. occurs (Haynes et al, 1984). UV-irradiation of adenovirus (this study) increased the progeny reversion frequencies in unirradiated normal fibroblast strain CRL1221 in a dose-dependent manner. The targeted reversion frequencies observed in unirradiated fibroblasts were always greater than those observed for intact virus progeny reversion frequencies obtained from either unirradiated or UV-irradiated fibroblasts. The increased targeted mutagenesis associated with a UV-irradiated viral probe suggests that the damaged viral DNA may induce EPR after viral infecton. The preinfection of lethally UV-irradiated HSV-1 in normal and XP fibroblasts suggested that exogenously damaged DNA was capable of further stimulating a

targeted increase. This may indicate that a UV-damaged viral probe in a similar manner could stimulate a targeted response (possibly EPR) that was subsequently involved in the repair of the infecting virus. Preirradiation of the host monolayers 24 hours before infection with damaged virus decreased the length of time until appearance of viable progeny when compared to unirradiated monlayers (Figure 1). The fact that pooled normals expressed only a small (but significant) UVEM response may indicate a strong competition in these assays between EFR and EPR processes to correct potentially lethal UV damage., The UV-dose dependent increase in reversion associated with UV-irradiaton of virus has been similarly described in a number of viral systems includng parvovirus H-1 (Cornelis et al, 1982) SV40 (Sarasin and Benoit, 1980; Taylor et al, 1982), adenovirus (Day and Ziolkowski, 1981; Piperakis and McLennan, 1984) and HSV-1 (Das Gupta and Summers, 1978; Lytle et al, 1980; Lytle and Knott, 1982; Lytle et al, 1982) for a variety of cell types and appears to be a generalized phenomenon.

III.e. Molecular Significance of UVEM Calculations

The lack of a unified nomenclature associated with mutagenic repair processes has allowed a variety of definitions as to what constitutes error-prone repair or enhanced viral mutagenesis. Some authors have defined EM with respect to the untargeted increase (Cornelis et al, 1981; Su et al, 1981; Cornelis et al, 1982), or targeted increase (Das Gupta and Summers, 1978; Mezzina et al, 1981; Sarasin et al, 1982; Takimoto, 1983) or by the ratio of targeted to untargeted increase resulting from preirradiation of the cells

(Sarasin and Benoit, 1980; Lytle et al, 1980; Day and Ziolkowski, 1981; Cornelis et al, 1982; Lytle and Knott, 1982; Taylor et al, 1982; Piperakis and McLennon, 1984). If enhanced mutagenesis is to be compared to ER phenomena then the operational definition of EM should be the equivalent of ER, where ER has been defined as the enhanced survival of UV-irradiated virus in irradiated cells divided by the survival of UV-irradiated virus in control cells (Equation 5). Similarly, EM is the increase in reversion frequency of UV-irradiated virus (compared to intact virus) in UV-irradiated cells divided by the increase in reversion frequency of UV-irradiated virus (compared to intact virus) in control cells (Equation 1). This is the equivalent of the ratio of targeted increase to untargeted increase. If these ratios are greater than 1 error-prone repair processes have increased the mutations at targeted rather than untargeted sites suggesting the existence of "hitchhiking" errors (Bridges, 1983; 1985) or "locally targeted" damage (Walker, 1984). "Hitchhiking" errors are hypothesized to result from an error-prone repair complex which create peripheral mutations in the sequences surrounding the targeted site as well as misreplicating the UV-induced premutational lesion. Normal fibroblasts may exhibit such "hitchhiking" errors considering that upon a 24 hour delay of adenovirus infection following cellular induction, the UVEM was 1.4 ± 0.2 (Table 5). Hitchhiking errors as indicate by UVEM values greater than 1 have been implied for SV40 (Sarasin and Benoit, 1980), and HSV-1 (Lytle and Knott, 1982). Other investigators have found SV40 UVEM values less than 1 in monkey kidney cells which indicated that error-free processes may contribute to ER phenomena in these cells

(Cornelis et al, 1980; Cornelis et al, 1981; Taylor et al, 1982) and has led some investigators to suggest that the untargeted increase responses may parallel (or contribute to) enhanced reactivation of virus (Cornelis et al, 1980; Cornelis et al, 1981). A small amount of hitchhiking error (UVEM) was evident when examining the kinetics of induction of revertants of intact or UV-irradiated H-1 virus grown in control or preirradiated human NB-E monolayers (Cornelis et al, 1982). This was evident by comparison of the slopes of H-1 viral mutation frequency vs UV dose to the virus for either control or UV-irradiated monolayers. A "mutator" activity present in preirradiated cells seemed to operate with a higher probability of error at some UV-damaged sites, than on intact portions of the same viral genome (Cornelis et al, 1982). It was concluded by these authors that induced mutagenesis of UV-damaged H-1 in preirradiated cells was close to the sum of: (i) the mutations induced indirectly in intact virus by an activated mutator; and (ii) the mutations that arose directly from the processing and/or replication of damaged viral templates.

A comparison of the kinetics of mutagenesis using UV-irradiated HSV grown in either preirradiated or unirradiated NB-E monolayers (Lytle and Knott, 1982), indicated that no untargeted increase occurred for intact HSV virus grown in preirradiated cells but enhanced targeted increases were found in UV-irradiated cells and may be due to "hitchhiking" errors possibly the result of an error-prone repair mode. The previous authors concluded that the lack of a HSV untargeted increase (as compared to parvovirus H-1 in the same cells which exhibit an untargeted increase) may be a function of the use of different DNA

polymerases by each virus, together with the different strandedness of their respective DNA's (Lytle and Knott, 1982; Cornelis et al, 1982). Alternatively, the large coding capacity of the HSV genome may encode a function that "turns on" or mimics the untargeted mutator response in some HSV infected cell lines such that cellular UV-inducing functions are masked.

The lack of an untargeted increase may depend on the host cell used since UV-irradiation of CV-1 monkey cells show a small untargeted increase (1.4x) for the growth of intact HSV (Lytle et al, 1980) whereas human fibroblasts strains (untransformed) show large untargeted increases (2-3 fold) for UV-irradiated HSV (Abrahams et al, 1984). As discussed previously, the later result may be a function of differences in the method of the $TK^+ \rightarrow TK^-$ mutation assay. The report of Lytle et al. (1980) gave no evidence of hitchhiking errors for UV-irradiated HSV in CV-1 cells since UVEM values less than one (0.95 at high moi of the UV-irradiated virus) or (0.70 at low moi of the UV-irradiated virus) were found and led to the suggestion that the increased EM may be dependent on multiplicity reactivation (Lytle et al, 1980). Takimoto (1983), using HSV as a probe of CV-1 cells, found a small untargeted increase for intact virus when the dose to cells exceeded 10 J/m^2. It may be possible that CV-1 cells are at least partially refractory in the ability to produce an untargeted increase of intact HSV mutagenesis following preirradiation of the cells.

Previous use of adenovirus as a probe of "hitchhiking" errors (represented by a UVEM greater than 1) in normal fibroblasts, HeLa cells or A498 cells did not reveal any increased kinetics of mutation

induction in preirradiated cells as compared to control cells over a dose range of 0-800 J/m^2 to virus and 5 J/m^2 to the cells (Day and Ziolkowsky, 1981; Piperakis and McLennan, 1984). These assays did, however, reveal untargeted increases using adenovirus as a probe.

Indication of a mammalian error-prone repair system (with UVEM values much greater than one) has been described using SV40 as a probe in CV-1 monkey kidney cells that were UV preirradiated (Sarasin and Benoit, 1980). The significance of this finding is in doubt since other investigators have found greatly reduced UVEM values using SV40 as a probe of BSC-1 cells (Cornelis et al, 1980; Cornelis et al, 1981) or CV-1 cells (Taylor et al, 1982). The reason for this discrepancy is not clear since all the above studies produced UVER of virus; however, in one study all the published reversion frequencies of intact virus were over estimations since no plaques developed at the non permissive temperatures at the low moi used (Sarasin and Benoit, 1980). This makes an accurate calculation of the untargeted increase impossible in the previous study which in turn prevents estimation of the true UVEM for SV40 progeny and thus may account for the discrepancies observed between laboratories.

III.f. Alternative UVEM Calculation

The UVEM calculations described above, equally weigh the effects of untargeted and targeted increases in determining if preirradiated cells exhibit viral EM phenomenan. This may be biased considering that in these assays, the mutagenesis (reversion) associated with a UV-irradiated virus was higher than that observed for

intact virus. As a result, targeted mutagenesis plays a more prominent role in viral EM phenomena since the targeted lesions contribute a greater proportion of the reversion events. This suggests an alternative definition of UVEM based on Equation 11 where the UV-induced reversion frequency in UV-irradiated cells is divided by the UV-induced reversion frequency obtained in uninduced cells. This measures the increased mutational response to damage observed at "targeted" sites corrected for the background reversion observed in either control or UV-irradiated host monolayers. A UVEM calculation of this type incorporates correction for the relative contribution of "targeted" and "untargeted" reversion frequencies. The validity of this calculation depends on the independent nature of the targeted and untargeted increase responses and assumes that the EPR associated with each process was additive (ie. the mechanism responsible for the loss of fidelity associated with the untargeted increase was not functional at UV-induced lesions). If the EPR process responsible for the untargeted increase was functional at targeted sites, then the mutagenic effects of cellular and viral UV-irradiation would be multiplicative. In this respect a UVEM calculated using the ratio of targeted to untargeted increase would be more appropriate. Examination of targeted vs untargeted increase has suggested that the untargeted response was maximally expressed after immediate viral infection while the targeted increase response was maximal after an infection delay of 24 hours in normal human fibroblasts. This suggests that UVEM values may be alternatively calculated using Equation 11 which emphasizes the response of control or UV-irradiated fibroblasts to targeted damage

Induced by UV-Irradiation of the viral probe.

UVEM calculation using equation 11 fail to take into consideration the mutagenic contribution of UVER which, if active, considerably decreases the amount of damage (lethal hits) in progeny obtained from UV-irradiated versus unirradiated host cells as illustrated by Figure 9. The presence of UVER even in the absence of an increased UV-induced RF of progeny for irradiated as compared to unirradiated cells implies that UVER is an error-prone repair process. If UVER were error-free a decreased UV-induced RF of viral progeny would be expected for UV-irradiated as compared to unirradiated fibroblasts. Table 18 indicates that for normal, CS and XPA fibroblasts, a higher UV-induced RF of viral progeny was observed in UV-irradiated versus unirradiated cells even though progeny obtained from UV-irradiated cells contained less lethal damage. On the other hand, a decreased UV-induced RF of viral progeny from UV-irradiated as compared to unirradiated cells was observed for the AT3BI and XP variant strains even in the presence of UVER (Table 18). Since decreased UV-induced RF values were observed in the presence of UVER, this implies that AT3BI and XP variants may possess an inducible error-free repair rather than lack an error-prone system whose activity is presumed through the presence of UVER. UVEM as calculated using equation 11 fails to correct for the survival increases (reflected by reduced lethal hits) induced by the activity of UVER; therefore, a more significant UVEM calculation is represented by equation 12 (UVEM$_2$). This calculation divides the UV-induced RF per lethal hit of progeny obtained from UV-irradiated cells by the UV-induced RF per lethal hit of progeny obtained from unirradiated

cells, thereby correcting for the enhanced survival observed during UVER.

The UVEM values of Table 5 (1.4 $\pm$ 0.2) were calculated using the ratio of targeted to untargeted increase (equations 1 and 4). If Equation 12 is used to calculate the UVEM for the data of Table 5, a significantly larger UVEM (2.4 $\pm$ 0.4) resulted (Table 18). This suggests there was significantly greater amounts of targeted mutation (reversion) in UV preirradiated human normal fibroblasts after correction for UVER and the "background" reversion of intact virus. Thus, UV-induced reversion in preirradiated fibroblasts was.2.4 fold greater than that found in control (unirradiated) cells. This again implies the existence of "hitchhiking" errors that may be the result of a damage specific EPR complex's ability to produce mutation not only at the targeted lesion sites but also in the flanking regions.

III.g. Error-prone Repair and the Mechanism of Human Carcinogenesis

The existence of an active EPR complex in normal human fibroblasts may have profound implications in the mechanism of human carcinogenesis. Point mutations have been described as an underlying cause of c-oncogene activation, especially in the case of the c-ras oncogene (Tabin et al, 1982). An EPR complex with both a damage specific and a random component (mutation into flanking regions) could account for the cellular activation of c-oncs after insult with physical (UV) or chemical DNA damaging agents. Support for the involvement of EPR in the activation of the ras oncogene has been suggested by the induction of mammary carcinoma in rats following a

single treatment of animals with the potent alkylating agent nitroso-methylurea (Sukumar et al, 1983). Molecular characterization of the transformation active genes (in the NIH 3T3 assay) revealed a targeted mutation at the 12th codon of the .H-ras-1 gene (G $\longrightarrow$ A transition) which was expected since NMU alkylates deoxyguanosine residues causing possible misparing during replication. Somatic mutation is not just a feature of the ras oncogene but has been described for a translocated c-myc in Burkitt's lymphoma (Rabbitts et al, 1983) and activation of the chicken c-myc has been shown to be due to somatic mutations as a result of intergration of avian leukosis virus (Hayday et al, 1984). An inducible EPR complex as suggested in this study may be a candidate as a mechanism of somatic mutation and c-onc activation in response to cellular DNA damage. An inducible mutagenic DNA repair may also be a mechanism to induce genetic variability (Echols, 1981) which may have consequence on the relative rates of evolution. EPR functions have even been hypothesized to be active during immunoglobulin rearrangement and may be responsible for the somatic mutations observed in the variable regions (Kim et al, 1981; Selsing and Storb, 1981). It is possible that an EPR as suggested by this study may play a role in all or some of the above processes.

IV.a. <u>Survival and UV-induced Reversion of Adenovirus after</u>
<u>Single Cycle Lytic Infections of Normal and Repair</u>
<u>Deficient Fibroblasts</u>

Studies on the kinetics of expression for UV-induced

mutagenesis and survival of mammalian viruses (and other microorganisms) have indicated that mutagenesis is a complex multistep process dependent on the interaction of cellular EFR and EPR responses to genomic damage (Haynes et al (1984) Walker, 1984). A number of assumptions must be made in any experimental study that uses the biological endpoints of mutagenesis and survival as a measure of radiation induced damage. Haynes et al, 1984 have suggested that classical hit/target theory which assumes that cell (or viral) killing or mutation is a direct and inevitable consequence of the accumulation of a minimal number of physical "hits" to the target, may present interpretive and theoretical difficulties. Hits are thought to be randomly distributed in uniformly irradiated homogeneous populations such that surviving fractions and mutation frequencies can be calculated using Poisson statistics and the average number of relevant physical hits per cell (or virus) can be calculated at a given dose. In classical hit/ target theory "hits" are generally assumed to be purely physical events whose number in the target volume, at all times after irradiation is proportional to the dose (Cramp, 1978). This assumption fails to consider any possible modification of the lesion during the expression of the biological endpoint (Haynes et al, 1984). What is actually observed is the survival or mutation among the irradiated population and these biological events are actually lethal hits or mutational hits. Biological hits are measured directly in terms of surviving fraction or reversion frequencies such that one biological hit is said to occur per cell (on average) at a dose that leaves a fraction of e^{-1} population unaffected (Haynes et al, 1984).

The above "repair theory" departs from classical theory in that a distinction is made between physical and biological hits such that a physical hit is either a potentially lethal or premutational lesion. Lethal hits are assumed to be unrepaired DNA lesions (Haynes, 1966). Mutational hits result from premutational lesions that escape EFR and are either repaired by EPR processes or tolerated by a bypass mechanism.

Another important consideration in the interpretation of the kinetics of reversion is the relative growth fitness of selectable mutants and non mutants. Depending on the assay system used, varying degrees of competition between adenovirus ts mutants and phenotypic wild type revertants may occur as the virus replicates.

Mutational dose-response curves have been classified on the basis of power (n) function by which mutation frequency increases with UV dose and the value of (n) can be calculated by determining the slope of a log-log plot of induced mutation (reversion) frequency per unit dose. Five main types of mutational responses have been identified: (i) linear where n = 1; (ii) quadratic where n = 2; (iii) multiphasic, such as linear-quadratic; and (iv) non integral where n equals some fractional power; and (v) n greater than 2 (Eckardt and Haynes, 1977). Under some conditions, UV-induced mutation curves saturate and decline after high doses (Haynes et al, 1984).

At low doses, UV-induced mutations in bacteria usually increase as the square of the UV-fluence (Witkin, 1976; Eckardt and Haynes, 1977; Fix and Bockwrath, 1983). UV-irradiated yeast similarily has demonstrated 2-hit kinetics (Lawrence et al, 1974), as have various

mammalian viral systems including SV40 (Cleaver and Weil, 1975; Sarasin et al, 1982) and adenovirus (Day and Ziolkowski, 1981). Three models have been proposed to account for the "2-hit" mechanism of UV-induced mutagenesis. A model that postulates the cooperation of two separate lesions at the target site (Doudney and Young, 1962; Mennigmann H.D., 1972; Bresler, S.E., 1975) has been proposed. A second theory suggests the selective inhibition of EFR but not EPR as UV dose increased (Bridges, 1975). The third suggestion that 2 pyrimidine dimers were needed, one to "signal" the induction of the "SOS" response and the other to serve as a premutational (targeting) lesion (Radman, 1975; Witkin, 1976; Fix and Bockrath, 1983). These theories can only account for 2-hit induction curves and are unable to account for the other different kinetic patterns observed when n is greater than 2 (Eckardt and Haynes, 1977).

In this study the induction of UV-induced revertants of Ad5ts36 and Ad5ts125 was examined in the normal human fibroblast strain CRL1221 such that a log-log plot of UV-induced reversion frequency vs UV fluence produced slopes (n values) of 2.5 $\pm$ 0.3 and 2.4 $\pm$ 0.5 for Ad5ts36 and Ad5ts125, respectively. These slopes appear to be intermediate (between n = 2 and n = 3), although given the large error in the estimates they could be considered as "essentially" quadratic (n = 2). Occasionally, induced mutation frequencies have been described with n values greater than 2 (Kolmark and Kilbey, 1968; Eckardt and Haynes, 1977; Haynes et al, 1984). Kolmark and Kilbey (1968), using alkylating agents in N. crassa, suggested that n values greater than 2 may be the result of additional factors such as the progressive

inactivation of an error-free repair system according to a strict kinetic formulation (Haynes, 1966). This may result in a linear induction curve (with n = 2) which bends upwards as the dose increases (Kolmark and Kilbey, 1968). The "bending" upward may be evident in the log-log plots of UV-induced reversion frequency vs UV-fluence for Ad5ts36 and Ad5ts125 (Figure 5) at high doses to the virus. Eckardt and Haynes (1977) suggest that the positive departure from linearity at high doses could be attributed in excision deficient yeast to stochastic dependence of mutation and killing which they termed delta-effects. In the case where delta was greater than 1, UV-induced mutation curves first rise linearly with dose, then become quadratic and ultimately increase at even greater powers of the dose (Eckardt and Haynes, 1977). A positive departure from linearity suggests that either (1) mutant and non mutant virus may have different survival fitness and/or (11) viral mutability may be dose-dependent and not constant. They further suggest that mutation and killing are not necessarily statistically independent processes (Eckardt and Haynes, 1977).

Other investigators have found that UV-induced reversion of Ad5ts2 produced an "n" value of 2.1 in an unirradiated A498 cell line or 1.9 in normal human fibroblast strain CRL1187 (Day and Ziolkowski, 1981). In a similar study using Ad5ts1 progeny from HeLa cells, 2.2 mutational hits were calculated from the data of UV-irradiated viral progeny grown in unirradiated monolayers (Piperakis and McLennan, 1984).

Other viral systems have indicated various "n" values for the

number of "hits" required to induce one reversion event following viral UV-irradiation. UV-irradiated SV40 has exhibited "n" values of 2.4 (Sarasin et al, 1982) or n = 2 (Cleaver and Weil, 1975) in unirradiated CV-1 while UV-irradiated H-1 produced an n value of 1.0 in unirradiated NBE human cells (as calculated from Cornelis et al, 1982). UV-irradiated HSV-1 produced n values of 1.1 in unirradiated NBE cells and 1.2 in unirradiated normal human fibroblasts (CRL1220), while XP variant cells (CRL1162) produced a "n" value of 2.3 (slope of a log-log plot of UV-induced reversion vs fluence; Lytle and Knott, 1982; Lytle et al, 1982). The latter result suggests that constitutive cellular differences may dramatically alter the number of "hits" required to induce a mutagenic event. The differences observed when comparing "n" values of Ad5 or SV40 (n = 2) to those of HSV (n = 1) were suggested to be the result of the different methods employed to score Ad5 or SV 40 mutants (reversion of ts mutants) vs a forward mutation assay (ICdR resistance) for HSV-1 (Lytle, et al, 1982). However, similar to HSV forward mutagenesis, H-1 assays measuring ts reversion induced by viral UV-irradiation also produced "n" values of 1.0. This suggests that the number of "hits" required to induce a mutagenic event is regulated by cellular and/or viral factors which are independent of the viral mutagenesis system employed.

In the present study Ad5ts36 and Ad5ts125 were examined for progeny survival in CRL1221 normal human fibroblasts such that UV-irradiation of virus produced a D_0 of 217 ± 20 J/m^2 for Ad5ts36 and a D_0 of 269 ± 20 J/m^2 for Ad5ts125. This compares to a D_0 of 204 J/m^2 calculated for irradiated Ad5ts2 infecting A498 cell monolayers after

infections at a mol of 1 (Day and Ziolkowski, 1981). The shape of the survival curves for Ad5ts36 or Ad5ts125 were essentially single hit, exponential inactivation kinetics whereas the data published for Ad5ts2 were "shouldered", suggestive of multihit inactivation kinetics (Day and Ziolkowski, 1981). This "shouldered" inactivation was indicated by the large D_{37} values ($D_{37} \simeq 500$ J/m^2) obtained from the progeny survival curves of UV-irradiated Ad2 in normal or A498 fibroblast cells (Day and Ziolkowski, 1981).

UV survival curves of Ad2 direct plaque formation demonstrated single hit, exponential inactivation kinetics such that the mean D_{37} of Ad2 plaque formation in 10 different normal human fibroblast strains had a value of $0.22 \pm 0.02 \times 10^3$ J/m^2 (Day, 1974). In pooled normal human fibroblasts that were not irradiated, a similar D_o of $0.23 \pm 0.06 \times 10^{-3}$ J/m^2 was observed for the HCR of UV-irradiated Ad2 Vag (Jeeves and Rainbow, 1983). These results were both similar to the Ad5 progeny D_o values described above and suggests that a similar target size exists for all three endpoints.

A plot of log UV-induced reversion versus log UV dose for both Ad5ts36 and Ad5ts125 indicated that at any given dose, Ad5ts36 produced a higher frequency of revertants following infection of normal strain CRL1221 with both mutants at similar moi. At a dose of 300 J/m^2 to virus 5x more revertants were scored for Ad5ts36 while at 1000 J/m^2 to virus 7.5x as many Ad5ts36 progeny mutants were detected. The reason for the increased sensitivity of Ad5ts36 is not clear; however, it may relate to differences in the revertable "target size" of the two mutants. The smaller size of the Ad5ts125 defective protein (72K, DNA

binding protein) as compared to the larger DNA pol (140K) which is defective in Ad5ts36, may limit the size of revertable target sites.

Using the survival data of individual progeny assays, it was possible to plot UV-induced reversion (in unirradiated cells) versus either progeny SF or the number of lethal hits for a number of normal and repair deficient human fibroblasts. A linear − log plot of the Ad5ts36 UV-induced progeny reversion versus progeny SF indicated the "power-function" nature of this curve (data not shown). Normal fibroblasts and the repair deficient syndromes AT, XP(group A), XP variant and CS appear to follow a power function described by the equation $Y = b (X)^n$ (see results). A rapid increase in RF in this curve occurs between a progeny surviving fraction of 10^{-3} to 10^{-4}. It appears that survival levels (in unirradiated cells) lower than 10^{-3}-10^{-4} enable the detection of larger increases in progeny RF per survivor when compared to survival levels above a progeny SF of 10^{-3}-10^{-4}. This can be observed in Figure 9 and may indicate that the failure of Day and Ziolkowski (1981) and Piperakis and McLennan (1984) to detect UVEM was the result of progeny survival above the "critical" (10^{-3}-10^{-4}) detection range.

IV.b.   Kinetics of UV-Induced Reversion of Adenovirus in Normal
        and Repair Deficient Human Fibroblast Strains

The difference between the two curves obtained in unirradiated and preirradiated fibroblasts is representative of the UVEM as calculated using the alternate definition (Equation 12). The data of Figure 9 clearly indicated that UVEM could only be detected beyond a

damage level of about 6 lethal hits to the progeny. UV-damage that resulted in fewer lethal hits to the progeny produced UV-induced reversion frequencies that were similar in control and preirradiated human fibroblasts. Calculation of UVEM by equation 12 and illustrated by Figure 9 strongly suggest the existence of a threshold for the detection of error-prone repair as assayed using Ad5ts36 progeny from normal and the repair deficient human fibroblast strains CS (GM2838), XP group A (GM710), XP variant (CRL1162) or AT (GM1588). Detection of UVEM phenomena using this system is facilitated by using viral UV-irradiations sufficient to induce 6 (or more) lethal hits in the viral progeny. Less damage as in the case of Day and Ziolkowski, 1981 or Piperakis and McLennan (1984) would make the detection of UVEM very difficult.

Kinetics of viral mutagenesis comparing UV-preirradiated normal and/or repair deficient fibroblasts have not been published; however, non-linear curves were found when HSV mutation frequencies (linear axis) were plotted vs lethal hits (linear axis) in unirradiated XP (group A), XP variant and normal fibroblast cells (Lytle et al, 1982). The plot of HSV mutation frequency (linear axis) vs lethal hits (linear axis) produced 2 component curves for normal and XPA fibroblasts whereas the curve for XP variant cells displayed a mutation frequency that increased as a power function (Lytle et al. 1982). Since HSV mutation frequency (linear axis) vs UV-dose (linear axis) produced linear curves for data using XPA or normals as hosts, the two component nature of the same curves plotted on a per lethal hit basis reflects the two component nature of the progeny survival curves. Furthermore,

UV-induced HSV mutagenesis was hypermutable on a per unit dose basis in XPA compared to normal, human fibroblasts but when corrected for survival by plotting per lethal hit no differences between these two strains was apparent (Lytle et al. 1978). This is in agreement with data obtained in this study and suggests that excision repair (which is deficient in XPA cells) is an error-free process. XP variant strains displayed abnormal UV-induced HSV mutability since a plot of UV-induced HSV mutagenesis (linear axis) vs UV-dose (linear axis) indicated a dose squared increase in mutation while normal or XPA fibroblasts displayed linear kinetics. In this study, normal and XPA fibroblasts displayed linear reversion kinetics when plotted in a similar manner; however, at a single UV-dose to the virus (1200 J/m^2), no difference was observed in the mutability of progency obtained from XP variant fibroblasts and from normal controls (Figure 7). Evidence for such a dose squared mutability using Ad5 as a probe (as detected with HSV) minimally requires a complete dose response reversion assay in XP variants. A log-log plot of HSV UV-induced mutation vs dose revealed that the least squared slope was 2.3 for irradiated virus grown in XPA and 1.2 for virus grown in normal fibroblast strains (Lytle et al, 1982). This further indicates that the mechanism of UV mutagenesis in XP variants may be different than normal. Moreover, a slope of 1.2 in normal cells for UV-irradiated HSV compares to slopes of 2.5 (Ad5ts36) and 2.4 (Ad5ts125) for UV-irradiated Ad5 in normal cell strain (CRL1221) (Figure 4). This indicates that HSV requires only 1 "hit" for the production of a mutational event whereas Ad5 requires between 2 and 3 "hits" for a reversion event in normal cells. The apparent differences

between Ad5 and HSV UV-induced mutagenesis may result from the different assay systems employed. HSV mutagenesis occurs as the result of a forward mutation $(TK^+ \longrightarrow TK^-)$ in a nonessential gene and will therefore encompass a wider spectrum of inactivating events (deletions, frameshifts, point mutation) whereas the Ad5 assay utilizes a reversion system in an essential gene presumably sensitive to only point mutations. Alternatively, HSV may code for genes involved in UV-induced mutagenesis that are not encoded by the smaller genome of adenovirus.

Deviations from unity in the slopes of mutation induction curves as observed in this study for Ad5 UV-induced reversion have been interpreted as indicating either (i) error-prone repair being switched "on" at specific damage levels resulting in increasing n values (greater than 2) when mutagenesis vs dose is plotted on a log-log axis or alternatively (ii) increased ability of UV-induced mutants to survive under the particular assay's selective conditions (Haynes et al, 1984).

Multicomponent dose response relationships have been observed for ionizing radiation induced chromosomal rearrangements in higher organisms (Brewer and Brock, 1968; Brewer et al, 1973; Brewer and Preston, 1974). Biphasic linear-quadratic dose response curves have been described for the generation of chromosomal aberrations by environmental mutagens (Neary et al, 1964). The underlying molecular mechanisms responsible for the linear portion of these curves has been hypothesized to be the result of a single track of ionizing radiation whereas the quadratic (2-hit) portion of these curves was the result of

the interaction of two tracks within a critical target distance along the rearranged chromosome (Neary et al, 1964). A similar process involving closely spaced (overlapping) UV-induced lesions within a critical target has been proposed for the generation of UV-induced mutations at high fluences in bacterial systems (Witkin, 1976). Furthermore, the generation of these mutations requires the active participation of a number of host gene products (Witkins, 1976; Walker, 1984). Although it is far from clear, closely spaced lesions (in a critical target) may be a "more mutable" substrate when compared to lesions that are widely spaced (as expected to occur at low UV fluences). Alternatively, closely spaced lesions may be a prerequisite for the induction of error-prone repair processes. The observation that large increases in UV-induced RF of adenovirus progeny from unirradiated or UV-irradiated human fibroblasts occurred only at low progeny survival levels (Figure 9) may suggest that mutational efficiency increased as the revertable target became saturated.

IV.c. UVEM and Multiplicity of Viral Infection (MOI)

The effects of moi upon parvovirus H-1 targeted and untargeted UV-induced mutagenesis suggested that at high moi (greater than 1), both targeted and untargeted mutations were decreased (Cornelis et al, 1982). These authors suggested that at high moi's, each infecting virus replicates a limited number of times, therefore is a very poor "target" for cellular or viral "mutator" functions. Increases in UVEM values for UV-irradiated HSV-1 in CV-1 cells were observed when larger moi's for UV-irradiated virus were used, thus leading to the suggestion

that UVEM may involve multiplicity reactivation (Lytle et al, 1980). At a moi of 0.2 for infecting UV-irradiated HSV, a UVEM of 0.7 was observed whereas at a moi of 2 (for UV-irradiated virus) a UVEM of 0.95 was observed (Lytle et al, 1980). In a separate study (Takimoto, 1984), increases in moi did not affect intact HSV-1 mutagenesis.

Using UV-irradiated SV-40 in either CV-1 or BSC-1 cell lines, UVEM was not observed in experiments conducted at low moi (Cornelis et al, 1980; 1981; Taylor et al, 1982). Furthermore, UV-irradiated Ad5 also failed to express UVEM in normal human fibroblasts at high (approximately 1.0) moi values (Day and Ziolkowski, 1981). Similarly high moi values (1.0 for UV-irradiated Ad5) in this study produced a UVEM significantly greater than one suggesting that cell and/or viral factors other than moi may determine the expression of UVEM of UV-irradiated viral probes. A further indication of the high multiplicity (moi approximately 1) in the experiments of this study is indicated by multiplying the average normal spontaneous reversions frequency (7.8×10^{-5}) by the number of cells infected (approximately 1×10^{6}) which results in a factor ≥ 1 for unirradiated virus.

V.a. Abnormal Expression of UVER and UVEM of UV-irradiated Adenovirus in Ataxia telangiectasia Fibroblasts.

The basic biochemical DNA-repair defect responsible for the AT phenotype remains to be defined; however, it is apparent that AT fibroblasts show a reduced mutation frequency compared to normal strains following X-irradiation (Arlett and Lehmann, 1978). This hypomutability was confirmed when gamma-induced mutations (to

6-thioguanine resistance) per survivor were found to be decreased in AT cells as compared to normals (Arlett,C.F., and Harcourt,S.A., 1980). When cellular UV-irradiation was used the AT cell strains had slightly elevated mutation frequencies compared to normals at high UV-doses and. It was concluded that AT fibroblasts may be defective in an "error-prone" repair of gamma damage (Arlett, 1980). It was of interest to examine the UVEM response of UV-irradiated adenovirus in AT fibrobasts following UV-irradiation since these cells may lack "error-prone" repair. Initial experiments using the AT strain AT3BI and an infection delay of 24 hours following cellular UV-irradiation revealed a hypomutability (compared to normals) associated with the targeted increase. This occurred in the presence of an untargeted increase and suggested that either a UV damage specific "error-free" repair system may be operative on UV damaged virus or alternatively an error-prone (lesion directed) repair system may be defective when compared to normal fibroblasts.

The decreased UVEM was observed in the presence of UVER values greater than one. This was the clearest indication that reactivation . may occur in the absence of UVEM. It appears that in AT3BI the two processes may be separate and regulated indppendently similar to the suggestion of Bresler et al (1978) for various E. coli mutants. As discussed previously, the decreased(UV-induced RF observed in UV-irradiated (as compared to unirradiated) AT3BI cells was evident even in the presence of UVER (Tables 13 and 18). If UVER is as mutagenic in AT as compared to normal cells, this implies an error-free mechanism may be active in AT. Bresler et al (1978) have similarly

suggested that the uncoupling of UVER (WR) and UVEM (WM) demonstrates that several repair systems are active in WR but only some of them are mutagenic.

Pooling of AT3BI experiments where viral infections occurred 24 hours after cellular UV-irradiation suggests that the low UVEM values (0.31 ± 0.1) were the result of an untargeted increase (1.7) significantly larger than the associated targeted increase (0.60, Table 13). The pooled UVER value (2.5 ± 0.9) indicated that when infections were delayed 24 hours following UV-irradiation of AT strains, enhanced viral reactivation was present although it was not accompanied by UVEM (0.31 ± 0.1). Few studies have examined the viral UVER/UVEM responses in repair-deficient human fibroblasts as compared to normal fibroblasts. Rommelaere (as communicated at the 9th Int. Cong. of Photobiology, 1984) has reported that AT fibroblasts are deficient in ER of UV-irradiated parvovirus. The absence of UVER in AT fibroblasts depends on the choice of viral probe used since UVER has been described for UV-irradiated HSV in AT host cells (Hellman, 1981). Viral UVEM values less than one (but similar in magnitude to that seen in ataxia) have been found in the monkey cell lines CV-1 (Taylor et al, 1982) and BSC-1 (Cornelis et al, 1980; Cornelis et al, 1981) using SV40 as a viral probe. Recently, a decreased EM (untargeted) for intact HSV-1 virus has been described for AT cells (as compared to normal) that was accompanied by normal UVER (Abrahams, as communicated at the conference "Mechanism of Mutagenesis: Impact on Carcinogenesis; Mettelwhir, France, 1985). Also described is a reduced ER of UV or X-irradiated parvovirus H-1 in UV or X-rayed AT fibroblasts (Hilgers, Chen,

Cornelis, Rommelaere et al, as communicated at Mechanism of Mutagenesis: Impact on Carcinogenesis; Mettelwhir, France, 1985).

Specific structural anomalies of chromosome 14 have been described for AT lymphocyte clones (Hecht et al, 1973; McCaw et al, 1975; Oxford et al, 1975; Rary et al, 1975; Aurias et al, 1980; 1983). The increased risk of lymphoid neoplasia in AT patients may be correlated with specific translocations and subsequent activation of cellular oncogenes (Klein, 1983). Specific chromosomal translocations involving chromosome 14 and 8 have been identified in many human Burkitt's lymphomas such that a c-myc gene is activated after translocation to the immunoglobulin heavy chain locus (Taub et al, 1982). It has been suggested that translocation of a c-myc (chromosone 8) into the transcriptionally active heavy chain locus (chromosome 14) results in either an altered or over expression of c-myc leading to a neoplastic state (Taub et al, 1982; Klein, 1983). It is extremely suggestive that AT may be cancer-prone due to a similar mechanism since a translocation identical to that typically seen in Burkitt's lymphoma was described for AT lymphocytes transformed by Epstein Barr virus (EBV) (Jean et al, 1979). It is also possible that the propensity for rearrangements of chromosome 14 may be responsible for the altered expression of immunoglobulin synthesis resulting in the immunodeficiencies seen in AT patients.

In light of the above implication of c-onc activation in AT cells, it is tempting to speculate that reduced UVEM may be the consequence of, or alternatively, a cause of oncogene activation in AT fibroblasts. Neoplastic progression has been proposed as occurring in

a stepwise, multistage process that involves somatic mutational events and/or epigenetic changes following carcinogen induced DNA damage (Echols, 1981; Cairns, 1981). Complete transformation of cells may be thought of as the final product of a process that requires the activation of specific cellular oncogenes with the coordinate expression of at least two cooperating oncogenes (Land et al, 1983; Rassoulzadegan et al, 1983). The increased susceptibility of AT patients to develop cancer may be a result of constitutive expression of an activated oncogene, therefore requiring only a single "hit" to allow complete cellular transformation. An alternative explanation for the triad of clinical manifestations associated with AT has been suggested in terms of abnormal DNA rearrangements associated with cellular differentiation during ontogeny and/or by the impairment of DNA repair (Breakefield and Hansen, 1983). These authors suggested that abnormal DNA rearrangements interfere with neuronal development and the progressive loss of neuronal function and cell number could result as secondary consequences of the abnormal neuronal development or from cumulative and unrepaired damage to neuronal DNA. Additional support for a defect in DNA rearrangement in AT can be justified since abnormalities associated with immunoglobulin synthesis have been identified in patients with this disorder. It may be suggested that the presence of a single defective gene locus (consistent with an autosomal recessive trait) may be at a site where DNA repair, immunoglobulin synthesis, neuronal development and a c-onc are closely linked. Alternatively, a defective DNA repair gene function(s) (recombinase?) needed to regulate the expression of oncogenes,

Immunoglobulins or neuronal development may be absent in AT.

Abnormal Vag expression of UVER responses were observed in AT fibroblasts infected immediately following cellular UV-irradiation but not after a delay of 24 hours following cellular induction (Figures 13,14). The lack of UVER observed in AT cells when infection was immediately following cellular UV-irradiation may be related to the finding that AT fibroblasts do not inhibit DNA replication following chromosomal DNA damage (Painter et al, 1982).

The dose response curves of ionizing radiation and DNA synthesis inhibition were found to be two component for normal cells and one component for AT cells (Painter et al, 1982). This has been interpreted as indicating that the steep (low dose) component of DNA synthesis inhibition by ionizing radiation (representing replicon initiation) is absent in AT cells, and the dose response for inhibition that does occur is parallel to the shallow (high dose) component for normal human cells (Painter, 1983). The shallow second component may represent a normal response to chain elongation for AT but when chain elongation is analysed in sucrose gradients it is apparent that elongation is radioresistant (Painter and Young, 1980; Painter, 1981; Painter, 1983). Since ionizing radiation blocks DNA chain elongation of normal but not AT cells and there are no differences in the damage induced between the two cell types, then this difference suggests that the radiation-induced lesions do not block chain termination and that in AT cells a mediator between DNA damage and chain termination is absent or nonfunctional (Painter, 1983).

Smith and Paterson (1983) using the DNA polymerase inhibitor

aphidicolin compared the rate of rejoining single strand breaks and the rate of removal of DNA lesions assayed as sites sensitive to the incising activity in extracts of M. luteus cells in gamma irradiated normal or AT fibroblasts. Further study of the AT defect using DNA specific bis-benzimidazole dye binding to DNA revealled that AT cells display increased ligand-induced DNA breakage and this appears to relate to a chromatin anomaly of enhanced accessibility (Smith, 1984). These authors concluded that human DNA polymerase alpha was not primarily involved in the repair of two classes of radiogenic DNA damage since the gamma-radiation responses of both AT and normal fibroblasts were not significantly affected by aphidicolin and suggested that a chromatin-associated abnormality in AT may be expressed irrespective of the presence or absence of aphidicolin. The non-involvement of pol alpha in the AT repair defect was suggested by Bertazzoni et al, (1978) who demonstrated that all three major DNA polymerases (alpha, beta and gamma) from AT cells were normal with respect to fidelity and efficiency of nucleotide reinsertion.

Although UV does inhibit replicon initiation in AT cells, the effect of UV on subsequent DNA elongation in AT is not known. Chromatin condensation may be altered in AT cells as compared to normal (Painter and Young, 1982) or AT may lack a DNA damage mediator or "signal" that interrupts DNA synthesis and thus enables constitutive DNA repair to occur (Painter, 1983). Such a "signal" mechanism has been considered essential in the activation of the "SOS" response in E.coli (Little, 1983; Walker, 1984). The lack of a hypothesized signal in AT cells may explain the hypomutability of these cells assuming that

mammalian fibroblasts have an equivalent of the bacterial "SOS" repair system. If this is the case, AT may be UV-hypomutable due to a lack of expression of a mammalian equivalent of the umuC gene product which has been found to be essential in bacterial UV mutagenesis (Walker, 1984). The lack of a DNA damage "signal" in AT may prevent the activation of a mammalian recA equivalent necessary for the expression of the "SOS" regulon. Aberrant induction of a mammalian recA recombinase in AT may account for the abnormal recombination dependent processes of neuronal development and immunoglobulin synthesis, observed in AT cells (Radding, 1981; Breakefield and Hansen, 1983). AT cellular hypersensitivity to gamma-irradiation and its corresponding hypomutability to gamma-irradiation suggests this disorder may represent a gamma-ray analog of bacterial recA⁻ strains and may mimic their response to UV-irradiation (Bridges, 1981). The UV-hypomutability and aberrant expression of UVER may add support to the analogy between AT and recA⁻ bacteria (Rainbow et al, 1983). A defect in UV-induced recombination of HSV was identified in AT, XP variant but not XP (A) fibroblasts when compared to normal human fibroblasts (Das Gupta and Summers, 1980). This supports the concept that a recombinase function possibly responsible for UVEM may be deficient in AT fibroblasts.

AT fibroblasts have not been extensively probed for DNA repair defects with mammalian viruses however Rainbow (1978) found reduced HCR of UV-irradiated adenovirus as compared to normals. AT fibroblasts were also found to be markedly deficient in gamma-ray ER of irradiated Ad2 (Jeeves and Rainbow, 1982).

No deficiency in the HCR of X-irradiated HSV was found in EBV transformed AT lymphoblastoid lines suggesting no defect in X-ray excision repair even though these cells were sensitive to X-irradiation in terms of colony formation (Henderson and Long et al, 1981; Henderson and Basilico, 1983). Similar to normals, AT cells express HCR of UV-irradiated H1 (Rommelaere, 1984) or HSV (Hellman, 1981). Since the AT defect may be due to an altered chromatin conformation resulting in reduced accessibility of repair enzymes to DNA (Smith and Paterson, 1983), it is possible that H1 or HSV DNA may replicate independently of host chromatin and thus allow normal access of repair enzymes.

V.b. Expression of UVER and UVEM of UV-irradiated Adenovirus

in Xeroderma pigmentosum (XP) fibroblasts

The cancer-prone nature of the XP autosomal recessive human disorder has led to the speculation that the reduced DNA repair exhibited by these cells may play a central role in producing somatic mutational event(s) leading to the increased risk of cancer (Straus, 1981). If this is the case, then the adenovirus UVER/UVEM responses may reflect abnormal error-prone repair processes associated with the XP repair defect. This has recently been suggested using HSV as probes of mutagenesis in normal, XP and XP variant fibroblasts (Abrahams et al, 1984). These authors found that viral EM (as measured by untargeted increase after cellular UV-irradiation) paralleled UVER in normal, XPA, XPC, and XPD fibroblasts while in XP variant cells, EM expression was delayed in comparison to viral ER.

XP25RO cells express a hypermutability of UV-induced mutation

frequency when compared to normal fibroblasts at a similar dose level (Lytle et al, 1982; this study). The kinetics of UV-induced reversion can be compared using cell strains of varying constitutive repair ability if mutation frequencies are plotted versus parameters that measure damaged virus survival. Target theory states that the number of hits (where a hit is a physical event) is strictly proportional to dose and a lethal hit (as measured by survival fractions) requires a defined number of physical events. In cases where the number of "events" needed is one then $SF = e^{-a}$ where (a) is the average number of lethal hits. Knowing the surviving fraction of any progeny assay allows the calculation of "a" and allows the comparison of normal and repair deficient fibroblasts that have different levels of constitutive repair. Comparison of the kinetics of log UV-induced reversion frequencies vs log lethal hits suggests that viral UV-mutagenesis in repair deficient fibroblasts (including XP25RO) was very similar to that seen in normal fibroblasts. This suggests that UV-induced viral mutagenesis in excision repair deficient fibroblasts was similar to normals when corrected for viral survival whereas, on a per unit dose basis, UV-irradiated Ad5 were hypermutable in XP as compared to normal. This is in agreement with the work of Lytle et al. (1982) using UV-irradiated HSV, thus supporting the conclusion that excision repair is error-free. UV-induced viral reversion plotted vs dose for XP25RO fibroblasts displayed a marked dose dependent hypermutability as compared to normals. XP fibroblasts themselves have also been found to be UV-hypermutable as a function of dose but were similar to normals when corrected by plotting cellular UV-induced reversion vs lethal hits

(Maher and McCormick, 1976). Furthermore, studies on cellular cytotoxicity to UV-irradiation have suggested that the rate of excision repair and the time until DNA replication determines the ultimate cytotoxic and mutagenic effects of UV-irradiation (Maher et al, 1979; Maher et al, 1982). Therefore, both mutagencity and cytotoxicity can be eliminated by excision repair supporting the suggestion that excision-repair is an error-free process (Maher and McCormick, 1976; Maher et al, 1979, Lytle et al, 1982).

Absence of a major error-free repair system in excision deficient XP25RO fibroblasts did not facilitate the expression of error-prone replication and/or repair as indicated by the absence of a UVEM of UV-irradiated Ad5 in these cells. In XP25RO cells, the targeted increase was smaller than the untargeted increase (Table 13) suggesting that an error-free system (other than excision) may be responsible for the increased fidelity associated with UV-damaged virus. A candidate for this process may be error-free multiplicity reactivation since UV-irradiated virus was infected at high moi ($\sim$1) and an analogous process has been described in bacteriophage T_4 (Yarosh, 1978).

The UVER of UV-irradiated or gamma-irradiated adenovirus has been previously studied in normal and XP fibroblasts using the "V" antigen assay (Jeeves and Rainbow, 1983, a, b). All XP strains tested (including XP25RO) express UVER although the UV doses to the XP cells which resulted in peak UVER were reduced as compared to normal strains (Jeeves and Rainbow, 1983 a, b). Similarly, UVER of UV-irradiated HSV has been observed at lower UV doses for XP strains as compared to

normals (Lytle et al, 1976; Lytle, 1978; Coppey and Menenzes, 1981; Ryan, 1983). An average UVER values in XP25RO of 1.3 ± 0.6 fell within the range of normal values for UV-irradiated Ad5 and suggested that in XP25RO, Ad5ts36 UVER was not accompanied by UVEM.

Equivalent UV fluences in normal fibroblasts as compared to XP would be expected to induce a similar number of lesions which would be repaired by available error-free excision processes with the ultimate level of mutagenesis dependent on the length of time cells had to repair (in an error-free manner) their DNA before entering S phase (Maher, et al, 1979; Konze-Thomas, 1982). The cancer-prone nature of this syndrome may be the result of a lower UV dose requirement to induce non-lethal but premutagenic lesions.

V.c. Induction of Ad5ts36 Reversion by Preinfection with Lethally
      UV-irradiated HSV-1

The data of Table 17 (HSV infecting normal strain GM2674 and excision deficient strain XP25RO) clearly demonstrated that preinfection 24 hours prior to Ad5ts36 infection with lethally UV-irradiated HSV could induce EM responses in both cell lines. Both cell strains were induced to express untargeted and targeted increases. The larger EM factors found under indirect (Table 17) as compared to direct (Tables 5 and 16) induction may be attributed to an elevated EM "signalling" mechanism associated with lethally UV damaged HSV-1. This "enhanced" signal responsible for EM may be the result of a trans-acting mechanism mediated by either a) "stalled" HSV-1 DNA replication resulting from numerous UV-induced replication blocks or b)

a virally coded protein or virally induced cellular protein(s). Presumably, indirect induction results in cellular mutagenic repair and/or replication that is equally active at untargeted or targeted sites as determined using unirradiated or UV-irradiated adenovirus as probes. Since direct UV induction in normal cells results in a larger EM of UV damaged virus as compared to unirradiated virus, this implies that the nature of the signalling mechanism may be different under direct as compared to indirect UV induction. Moreover, direct but not indirect UV-induction is able to mediate ER of UV-irradiated adenovirus suggesting that under indirect induction, the EM signalling mechanism may be different. This suggests that chromosomal damage resulting from direct UV-irradiation may be necessary to mediate the UVEM or ER phenomena observed in human cells.

ER of directly UV-irradiated mammalian viruses has been similarly described in a number of mammalian host cell systems following direct treatment with DNA damaging agents including UV (Lytle, 1978; Rainbow, 1981; Defais et al, 1983). Also, EM has been described for a variety of intact or UV-irradiated viral probes after direct UV-irradiation of host cells (Das Gupta and Summers, 1978; Lytle et al, 1980; Cornelis et al, 1980, 1981; Sarasin and Benoit, 1980; Lytle and Knott, 1982; Cornelis et al, 1982). Indirect activation of a mammalian cellular mutator responsible for EM has also been observed since preinfection of human or rat cells with UV-irradiated SV40 or parvovirus H-1 increased the mutagenesis of intact H-1 (Cornelis et al, 1981; Cornelis et al, 1982). Similarly, human or rat cells transfected with UV-irradiated a) double stranded DNA (calf thymus), b) closed

circular double stranded DNA (SV40) or c) closed circular single stranded DNA (φX174) enhanced the ability of recipient cells to mutate intact H-1 (Dinsart et al, 1984). In this study, preinfection with lethally UV-irradiated HSV-1 similarly enhanced the ability of infected cells to mutate not only intact adenovirus but UV-damaged adenovirus as well.

Enhanced mutagenesis of UV-irradiated bacteriophage has also been reported in bacterial cells which have taken up UV-irradiated episomes (George et al, 1974). Unlike in human fibroblasts infected with UV-irradiated HSV-1, conjugation of episomes from a UV-irradiated bacterial host to an unirradiated recipient mediates ER of UV-irradiated phage (George et al, 1974). This suggests that the mechanism of indirect induction may be different in bacterial cells as compared to human fibroblasts. Since indirect induction is an SOS dependent process in E.coli (Bailone et al, 1984), there may not be a totally analogous SOS-like process in human cells. Furthermore, indirect induction of SOS by intact or UV-irradiated phagemid miniF in E.coli requires the presence of the lynA locus on miniF in addition to host recA and lexA alleles. (Bailone et al, 1984; Brandenberger et al, 1984.) It has been suggested that lynA- coded protein(s) possibly involved in plasmid segregation may be required as an inducing signal (Bailone et al, 1984). A similar process dependent on protein synthesis from lethally UV-irradiated HSV-1 cannot be ruled out. UV-irradiated phages P1 and M13 (D'Ari and Huisman, 1982), unirradiated P1 miniplasmids (Capage and Scott, 1983) that carry specific alterations in their origins of replication, and plasmids pBR322 and

sex factor F in lexA (Def⁻) cells (Ennis et al, 1985) all stimulate SOS responses. Since UV-irradiation further enhances mutagenesis in lexA (Def⁻) cells carrying pBR322 or sex-factor F it has been suggested that some plasmids (or UV-irradiated phage) may mimick a small subset of the total population of SOS inducing signal(s) (Ennis et al, 1985). Lack of ER (in the presence of EM) following preinfection of human cells with UV-irradiated HSV-1 may also be attributed to a partial "SOS-like" induction mediated by only a fraction of the total population of inducing signal(s) present in directly UV-irradiated human cells.

V.d. Expression of UVER and UVEM of UV-irradiated Adenovirus

in XP Variant Human Fibroblasts

A reduced UVEM (0.52 ± 0.2) for UV-irradiated adenovirus was detected in pooled experiments using XP variant fibroblasts (Table 13) as compared to that observed for normals when viral infections were delayed 24 hours after cellular irradiation. This was similar to the result obtained for AT3BI and suggests that XP variants may lack an error-prone repair system or posses an inducible error-free system. These cells (XP variant) display UVER values similar to normals which increases the survival of UV-irradiated virus in pre-irradiated cells (Table 13), thereby reducing the number of lethal hits in progeny obtained from UV-irradiated as compared to unirradiated cells. This UVER is accompanied by a reduction in the UV-induced reversion frequencies in pre-irradiated XP variant cells (Table 18). Assuming that the process of UVER is similar (as mutagenic) in XP variant cells as compared to normal, this implies that XP variant cells may posses an

error-free system which does not interfere with UVER. If potentially
lethal lesions are also premutational lesions then correction by an
error-free system would be expected to enhance survival (and produce an
apparent UVER) while reducing mutagenesis. Since enhanced levels of
UVER (10 fold) have been detected for UV-irradiated HSV in XP variant
as compared to normals (Coppey and Menezes, 1981), this suggests that
such an error-free system may be more efficient in correcting
potentially lethal damage when compared to UVER processes found in
normal cells. Elevated UVER values were not found for UV-irradiated
Ad2 using the Vag assay (Jeeves and Rainbow, 1983) whereas a 2 fold
increase in UVER was found for 1 XP variant strain using UV-irradiated
HSV (Ryan, 1983). This suggests that differences in the Ad2 assay
system may inhibit the detection of elevated UVER in XP variant
strains. Support for an "inducible" error-free system operative in XP
variant cells can be found in the work of Abrahams et al. (1984) which
describes maximal UVER values occurring concomitant with the minimal EM
values for UV-irradiated HSV virus infected 24 hours after cellular
irradiation. An inducible error-free repair that alleviates lethality
without accompanying mutagenesis may account for this data.

UVER values for UV-irradiated Ad2 were found to be decreased in
XP variant strains as compared to that in normal strains using the Vag
assay (Jeeves and Rainbow, 1983b). A reduced (but not significantly
reduced) UVER was found for UV-irradiated Ad5 progeny in this study
(Table 13).

XP variant cells lack normal PRR mechanisms (Cleaver et al,
1981) and also express cellular UV hypermutability when measured on a

per survivor basis (Maher et al, 1976). This implies that deficient
PRR allows the expression of error-prone repair processes. Such
hypermutability was not observed in this study calculated on a per unit
dose basis (Figure 7) or when corrected for survival (Figure 8) and
compared to that expressed in normals. The lack of an abnormal
UV-induced Ad5 mutagenesis in unirradiated XP variant monolayers as has
been observed for UV-irradiated HSV (Lytle et al, 1982) may be the
result of the choice of an inappropriate dose (1200 J/m^2) to virus (as
discussed previously). Hypermutability of UV-irradiated Ad5 may
possibly be detected at higher UV-doses to the virus. The expression
of an inducible error-free replication and/or repair mode following
cellular UV-irradiation may account for the aberrations observed for
UVEM in this study and may contribute to the elevated levels of UVER
for UV-irradiated HSV found in XP variants as compared to normals
(Coppey and Menezes, 1981). Furthermore, the maximal UVER of
UV-irradiated HSV was concomitant with the minimal EM of XP variants
(Abrahams et al, 1984) suggesting that a process (possibly error-free)
contributing to the enhancement of fidelity (low EM) may also be
responsible for UVER in XP variants. The previous authors suggest that
ER and EM are transiently (time course dependent) expressed phenomena
operative in both normal and repair deficient cells, and both (ER and
EM) may be triggered by the same signal but can be expressed
independently (as in XP variant). Moreover, Abrahams et al. (1985; as
communicated at the conference Mechanisms of Mutagenesis = Impact on
Carcinogenesis) have suggested that the ability of cells to express ER
is positively correlated with sensitivity to cancer induction. In

addition, this study suggests that the ability to express reduced UVEM of UV-irradiated adenovirus may be a feature of fibroblasts obtained from cancer-prone individuals (AT, XP variant and to a lesser extent XPA) when compared to that observed for normal fibroblasts. It is tempting to speculate that abnormally low UVEM of UV-irradiated Ad5 may be the consequence of or alternatively the cause of oncogene activation in XP (similar to that previously suggested for AT).

It has been suggested that UV irradiation of mammalian cells can induce "SOS" - like processes reflected by enhanced reactivation of UV-irradiated viruses (similar to Weigle reactivation in bacteria) which, in the case of normal fibroblasts, may be accompanied by enhanced viral mutagenesis. Since SOS - like functions have a possible involvement in carcinogenesis (Radman, 1980; Echols, 1981) with a central role for proteolysis in SOS expression, it is not surprising that the cells derived from patients with the cancer-prone XP syndromes have been found to express the serine protease plasminogen activator (PA) in response to UV damage (Miskin and Ben-Ishai, 1981). PA induction occurred in all XP homozygotes, XP heterozyotes and human amniotic cells but not in normal human fibroblasts, suggesting that PA induction is associated with deficient DNA repair and may represent a eukaryotic SOS - type response (Miskin and Ben-Ishai, 1981) possibly linked to carcinogenesis.

The clinical symptoms and mutation induction in XP variants is similar to that in excision deficient XP patients and cell lines and it has been suggested that multiple biochemical alterations may underline a similar set of clinical and cellular phenomena (Cleaver et al, 1979).

V.e. Adenovirus UVER and UVEM Responses in Cockayne's Syndrome

Cockayne's syndrome (CS) fibroblasts like XP variant cells, fail to recover DNA synthesis following cellular UV-irradiation. While XP variant cells continue to replicate UV-irradiated DNA in short lengths, with replication forks stopping at almost every dimer, CS, on the other hand, replicates past dimers with increasing efficiency, but replicates in decreasing numbers of clusters (Cleaver et al, 1982). Furthermore, CS cells display large increases in the accumulation of long lived spontaneous DNA breaks when compared to that observed in normal controls (Squires and Johnson, 1983). These authors suggest that CS cells express some defect in the post incision step(s) of excision repair, possibly involving ligase.

Similarly, a defect in excision repair has been suggested in CS cells by the observation that CS cells express reduced levels of HCR for UV-irradiated Ad5 or Ad 2 (Day et al, 1981; Rainbow and Howes, 1982). CS cells have been found to be hypermutable when UV-induced mutation frequency to 6-thioguanine was compared to normals (Arlett, 1980). A similar hypermutability was not observed among Ad5ts36 progeny when UV-induced reversion frequencies were plotted as a function of UV dose or lethal hits (Figures 7 and 8). The magnitude of UVEM and UVER were very similar to that observed for normals. This agrees with the similar UVER values obtained for UV-irradiated Ad2 in CS as compared to normal human fibroblasts (Jeeves and Rainbow, 1983b). The apparent lack of any abnormality among the various parameters of UV-mutagenesis in CS cells obtained from patients who are not

cancer-prone is suggestive evidence that abnormal UV mutagenesis may be a hallmark of human carcinogenesis. Since all the fibroblast strains from cancer-prone syndromes (AT, XPA, XP variant) demonstrated aberrant patterns of UV-mutagenesis, it is possible to speculate that aberrations in pathways for UV-induced viral mutagenesis are in some way related to the predisposition of these syndromes to cancer. It must be realized that the apparent lack of elevated levels of cancer in CS patients may be the result of the relatively short life span of these patients which could prevent the detection of neoplasia of delayed onset (Bridges, 1981).

VI. DNA Damage and Biological Expression of Human Adenovirus Following Gamma-Irradiation at 0 and -75°C

The radiosensitivity of several viral functions of Ad2 were examined after gamma-irradiation at 0°C. The most sensitive viral function was that of plaque formation on human KB cells with a D_o value of 99 $\pm$ 14 Krads. This value is more that four-fold less than the D_o value of 468 $\pm$ 24 Krads calculated from the data of Rainbow and Mak (1972) for Ad2 gamma-irradiated at -75°C and indicates a considerable protective effect at the reduced temperature. Similar radio protective effects of reduced irradiation temperature have been reported for herpes virus (Lytle et al, 1972; Zamansky and Little, 1982).

The least sensitive viral function after gamma-irradiation at 0°C was that of viral adsorption to KB cells with a D_o value of 972 $\pm$ 118 Krads. This loss of adsorption presumably results from protein damage to the viral capsid rather than damage to the viral genome since

viral adsorption does not require expression of viral DNA. Thus under these conditions, about 10% of the inactivation was from loss of cell attachment due to protein damage, the remaining inactivation resulting from damage to the viral DNA. This is in contrast to inactivation of Ad2 under frozen irradiation conditions (at -75°C) where approximately 25% of viral inactivation resulted from loss of cell attachment due to protein damage. Thus loss of adsorption contributes to a greater extent to loss of infectivity following irradiation at -75°C as compared to 0°C.

Both viral DNA and protein can be damaged by ionizing radiation (Freifelder, 1965; 1966; Taylor and Ginoza, 1967; Boyce and Tepper, 1968; Van der Schans and Blok, 1970; Johansen et al, 1971; Coquerelle and Hagen, 1972; Clarkson and Dewey, 1973; Rainbow and Mak, 1972). Previous reports from this laboratory have shown that after irradiation of Ad2 at -75°C, 0.5 S.S. breaks/rad/10^{12} daltons and 0.01 D.S. breaks/rad/10^{12} daltons were induced in the viral DNA (Table 21). This compares to 1.7 S.S. breaks/rad/10^{12} dalton and 0.026 found in the present study, after irradiation at 0°C. Irradiation of Ad2 at 0°C in the liquid state increased the S.S. breakage per rad more than 3x and increased the D.S. breakage per rad about 2.6x when compared to irradiation in the frozen state at -75°C (Table 21). The S.S./D.S. breakage/rad ratio increased (from 50 at -75°C) to 65 at 0°C. This was due primarily to a greater increase in S.S. breakage than D.S. breakage at 0°C as compared to -75°C.

The data presented in Table 21 indicates that more S.S. and D.S. breaks/rad were induced after irradiation at 0°C compared to -75°C

but a smaller (or equal) number of S.S. and D.S. breakage/lethal hit was observed at 0°C vs -75°C. This suggests that gamma damage may be quantitatively and qualitatively different at 0°C as compared to -75°C. When the breaks/lethal hit are greater than one, such as the values for S.S. breaks/lethal hit found in this present study, it suggests that either the breaks are not lethal, or that they are repaired (or tolerated) possibly through a bypass mechanism. Single strand breaks/lethal hit have been found to be greater than one in many phage systems and loss of plaque forming ability has been correlated with double strand breakage (Freifelder, 1965; 1966, Table 23).

Investigators have also found "other" nucleotide damage as a major component of lethality in phage ϕX174 and PM2 after low LET irradiation (Christensen et al, 1972; Van der Schans, 1973). Nucleotide damage has been estimated to account for as much as 87 percent of the inactivation of single stranded PM2 and greater that 90 percent for ϕX174 (Christensen et al, 1972). Single-strand breakage accounted for only 8.5 percent of the lethality and double-strand breakage accounted for the remaining 4.5 percent of the lethality of PM2 DNA gamma-irradiated in oxygenerated solution (Christensen, 1976). Low LET irradiation of T_4 and T_7 have suggested a much larger contribution of double strand breaks to inactivation (Freifelder, 1965; 1966, Van der Schans and Blok, 1970). This suggests that the repair of other double stranded DNA damage in T_4 and T_7 is much more efficient than in single stranded PM2 or ϕX174. In this study D.S. breaks/lethal hit was found to be 0.065 $\pm$ 0.020 (Table 21). This value was much less than one and indicated that D.S. breakage did not play a

major role in biological inactivation of Ad2 plaque forming ability. Gamma-irradiation at -75°C produced a signficant decrease in the number of D.S. breaks but did not significantly increase the number of D.S. breaks/lethal hit. The S.S. breakage rate similarly increased in Ad2 DNA when gamma-irradiation was at 0°C as compared to -75°C (Table 21). The S.S. breakage/lethal hit was not significantly different between the gamma-irradiations at the two temperatures.

S.S. breaks which are close enough together but in opposite DNA strands may induce D.S. breakage. This does not seem to be the case since the D.S. breakage increased linearly with dose (Figure 20). If S.S. breakage was contributing to D.S. breakage a non-linear dose squared relationship would result (Hagen, 1967). The probability of two independent S.S. breaks occurring close enough together and inducing a D.S. break is too small to account for the observed D.S. breakage over the range of doses used in these experiments. Published data on strand breakage is more extensive in bacteriophage than in mammalian viruses and Table 23 lists the S.S. breakage rates for ionizing radiation in various phage and Ad2. In this study Ad2 had a S.S. breakage rate of 1.7 S.S. breaks/rad/10^{12} daltons which compares most closely to the published rates for T_4 (1.6 S.S. breaks/rad/10^{12} dalton) and T_7 (1.5 S.S. breaks/rad/10^{12} dalton) after irradiation under liquid conditions. S.S. breakage of phage lambda (RF) was determined during superinfection and covered a range from 0.5 - 4.7 S.S. breaks/rad/10^{12} daltons. The S.S. breakage of the replicative (DS) form of phage lambda was increased in the presence of 0_2 as compared to values obtained in the presence of N 74 had 4.9 - 7.6

S.S. breaks/rad/10^{12} daltons using an alkaline assay which was higher than the value found for Ad2 in this study. Ad2, however, had more S.S. breaks/lethal hits (4.4) as compared to 1.5 S.S. breaks/lethal hit for ØX174. This indicates that Ad2 can either tolerate more S.S. lesions than ØX174 or that greater proportion of "other" damage contributes to lethality in ØX174 than in Ad2. Using a neutral assay the S.S. breaks/rad/10^{12} dalton is lower in ØX174 than that found in the alkaline assay. The S.S. breaks/lethal hit was also lower using the neutral sucrose assay.

The S.S. breakage values of phage lambda decreased in the presence of histidine as compared to the value obtained in buffer alone. Increased breakage seen in buffer was probably due to a lack of protection from diffusable free radical damage by the organic compound histidine. This was also seen for T_4; however, the S.S. breakage in buffer alone, was much higher (18 S.S. breaks/rad/10^{12} dalton) as compared to irradiation in buffer supplemented with the organic compounds histidine and cysteine (1.9 - 2.9 S.S. breaks/rad/10^{12} daltons). The breakage rates obtained in the presence of radioprotective organic compounds were similar to the S.S. breakage rate found in this study. The low breakage rate (1.7 S.S. breaks/rad/10^{12} dalton) seen in this study may, in part, be due to the presence of glycerol during irradiation which may serve a radioprotective function. Under similar conditions in the absence of glycerol, the S.S. breakage rate of Ad2 was found to increase to 10.9 S.S. breaks/rad/10^{12} daltons (Palcic and Skargard, 1975). It is apparent that glycerol may have a radioprotective function when Ad2 is

gamma-irradiated at 0°C.

It can be seen from Table 23 that the S.S. breaks/lethal hit for T_7 and T_4 were higher than the values listed for Ad2. This indicated that S.S. breaks may not contribute as much to lethality in T_7 and T_4 as in Ad2. It is also possible that another lesion contributing to lethality, is more prominant in Ad2 as compared to T_4 or T_7. Alternatively, a higher S.S. breaks/lethal hit may be found in bacterial systems if S.S. breaks are repaired more readily than in mammalian systems.

The decrease in S.S. breakage can be attributed to the known radioprotective action of glycerol. It has been previously found that the production of S.S. breaks which depend on radiation chemical events involving free radicals can be significantly reduced in the presence of radical scavengers (Vos and Kaalen, 1962; Roots and Okada, 1975). The Ad2 virus suspension used for the S.S. and D.S. break analyses had a glycerol concentration of about 10% (v/v) or 1.4 M which produced a S.S. breakage rate 6 fold less than that reported by Palcic and Skarsgard (1975). Increased S.S. breakage of Ad2 DNA in the latter study may be due in part to oxygen enhancement during the irradiation procedure. Gamma-irradiation of Ad2 in the frozen state (Rainbow and Mak, 1972) also reduced the S.S. breakage rate 3 fold from that found in the present study which used liquid conditions. Gamma-irradiation in the frozen state will inhibit free radical diffusion and the lower S.S. DNA breakage rate is probably due to a smaller contribution of indirect effects. Access of the viral DNA to water radiolysis products allows for the possible induction of a different spectrum of lesions as

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YIELDS OF SINGLE STRAND BREAKAGE BY ALPHA AND GAMMA-RAYS IN DOUBLE
STRANDED DNA PHAGE AND VIRAL GENOMES

IRRADIATION OF	CONDITIONS OF IRRADIATION	SS BREAKS/RAD/ 10^{12} DALTONS	SS BREAKS/ LETHAL HIT	REFERENCE
□ RFφX174	AIR; -196°C □	1.2 (N)*	0.75	(Taylor and Ginoza, 1967)
□ RFφX174	AIR; 10°C □ radioprotective broth	4.9-7.6 (Alk)	1.5	(Van der Schans and Blok, 1973)
λ	O_2; 0°C	3.5-4.7 (Alk)		(Johansen et al, 1974)
	N_2; 0°C	0.9-1.1 (Alk)		
λ	O_2; 0°C	4.4 (Alk)		(Johansen et al, 1974)
	N_2; 0°C	1.3 (Alk)		
λ	O_2; 0°C	1.4 (Alk)		(Boyce and Tepper, 1968)
	N_2; 0°C	0.5 (Alk)		
α	AIR; 0.01M Po_4 buffer	4.5 (F)		(Friefelder, 1966)
	AIR; 10^{-3}M HISTIDINE	2.6 (F)		
T_7	O_2; SSC pH8.0	1.5 (Alk)	12.5	(Van der Schans and Blok, 1970)
T_7	N_2; 10^{-2}M Po_4 buffer	18 (F)	13	(Friefelder, 1965)
	N_2; 10^{-3}M HISTIDINE + CYSTEINE	1.9-2.9 (F)	9.3-14.2	
T_4	O_2; 100mM thiourea	1.6 (alk)	13	(Van der Schans and Blok, 1970)
	O_2; SSC pH8.0	1.2 (Alk)	10	
	AIR; 100mM thiourea	1.6 (Alk)	13	
Ad2	O_2; 0 C; PBS+	10.9 (Alk)		(Palcic and Skarsgard, 1975)
	N_2; 0 C; PBS	2.9 (Alk)		
Ad2	AIR; -75°C; CsCl + TBSΔ	0.5 (Alk)	7.9	(Rainbow and Mak, 1972)
Ad2	AIR; 0°C; CsCl + TBS	1.7 (Alk)	4.4	(this study)

* (Alk) = Alkaline sucrose assay
  (N) = Neutral sucrose assay
  (F) = Formaldehyde assay

+ PBS = phosphate buffered saline
Δ TBS = TRIS Buffered saline
□ = irradiated in vivo

compared to DNA irradiated in a frozen state. Induced nucleotide base damage by water radiolysis products has been found in gamma-irradiated phage T_7 (Feldberg and Carew, 1981) and RFϕX174 (Van der Schans and Blok, 1973). It has also been suggested that gamma-ray induced alkalai-labile sites result from not only the loss of a base but also from sugar damage in ϕX174 DNA gamma-irradiated in the liquid state (Lafleur et al, 1978). It is possible that base or sugar damage contributing to lethality is more prominent during gamma-irradiation at 0°C and that this damage may be repairable in XP25RO. Additional DNA damage induced at -75°C may have a "UV-like" nature since gamma-irradiated Ad2 (at -75°C) cannot be reactivated by the excision deficient XP25RO to as great an extent as Ad2 gamma-irradiated at 0°C (Table 22, Figure 19).

When the data for normal and XP25RO fibroblasts were pooled, the D_o of Vag formation of gamma-irradiated Ad2 in XP25RO expressed as a percentage of that obtained on normal fibroblast strains was 88 ± 3 (for gamma-irradiation at 0°C, 4 experiments) and 57 ± 17 (for gamma-irradiation at -75°C, 3 experiments). Survival of Ad2 gamma-irradiated at -75°C as measured by the D_o of Vag survival was therefore significantly decreased in XP25RO as compared to pooled normals. This is similar to previously published work using the Ad2 Vag assay where XP strains from complementation groups A, B, C and D resulted in Vag survival which was about 50 percent of that in the normal strains used (Rainbow and Howes, 1979).

It has been reported that gamma-irradiation of HSV-1 at 0°C decreased the survival of the virus in 15 human fibroblast strains when

compared to the survival of HSV gamma-irradiated at -75°C (Zamansky and Little, 1982). For normal strains they reported average D_o values of 114.9 Krads for virus gamma-irradiated at -75°C and 41.4 Krads for virus gamma-irradiated at 0°C. These compare closely to the previously published D_o values of 43 Krads (liquid state) and 150 Krads (frozen state) obtained for X-ray inactivation of HSV (Lytle and Goddard, 1979; Lytle et al, 1972). The HSV D_o value in normals obtained using virus gamma-irradiated in the frozen state was about 2.8x the D_o value obtained with virus gamma-irradiated at 0°C (Zamansky and Little, 1982). With Ad2 the D_o value for normals using Ad gamma-irradiated at -75°C was approximately 4.3x the D_o obtained with virus gamma-irradiated in the liquid state. This difference between HSV and Ad2 may be due in part to the different assay systems used since the HSV experiments used plaque formation as an end point whereas the Ad2 data was determined using the Vag assay.

Plaquing data for Ad2 gamma-irradiated under frozen conditions gave a D_{37} value of 460 Krads as assayed in KB cells (Rainbow and Mak, 1972). In this study using Ad2 gamma-irradiated at 0°C a D_o value of 99 Krads was found when assayed in KB cells. Frozen conditions therefore yielded a D_o value 4.6 times those found in the liquid state. Herpes virus assayed in CV-1 monkey cells using virus X-rayed at room temperature produced a D_o value of 150 Krads (Lytle and Goddard, 1979), while normal human cells produced a D_o value of 43 Krads using HSV gamma-irradiated in the frozen state. Under frozen irradiation conditions, X-ray damaged HSV gave a D_o value 3.5x the D_o observed for liquid irradiation conditions. As mentioned above, gamma-irradiation

In the frozen state and subsequent assay on normal human fibroblasts produced a D_o value 2.8x that found for virus gamma-irradiated in the liquid state (Zamansky and Little, 1982). HSV has a genome MW of 100 x 10^6 while Ad2 has a MW of about 23 x 10^6 and the GC contents of the two are similar (Flint, 1980). On the basis of the DNA target size alone, it would be expected that HSV would be about 4x as sensitive as Ad2 to gamma-irradiation assuming that both viruses utilized the same percentage of their genomes for plaque formation. This also assumes that the protein damage (which may cause loss of adsorption) was equal for the two viral systems. The difference in D_o values of Ad2 and HSV gamma-irradiated under frozen (direct) condition was about 4x (460 Krads/115 Krads). This would be expected based on relative DNA target size alone if the DNA is the principle target for biological inactivation. The difference in D_o values of Ad2 and HSV gamma-irradiated under indirect (liquid) conditions is approximately 2.4x (99 Krads/41.4 Krads). This difference was smaller than expected and suggests that HSV may be able to repair the spectrum of lesions induced under indirect conditions to a greater extent than those lesions induced under direct conditions as compared to adenovirus. There may be a dependence on viral coded gene products which can selectively repair the DNA damage induced by gamma-irradiation at 0°C. HSV induces the synthesis of several enzymes involved in deoxyribonucleotide metabolism and viral DNA synthesis (Huzar and Bacchetti, 1981; Francke and Garrett, 1982). It is possible that HSV codes for or induces a repair function which is able to selectively repair damage induced at 0°C. This hypothesis is not unprecedented

since bacteriophage T_4 codes for or induces many enzymes involved in deoxynucleotide metabolism, DNA synthesis, thymidine dimer repair and UV mutagenesis (Pawl et al, 1976; Yarosh, 1978).

Correlation of molecular damage and biological inactivation of Ad2 DNA gamma-irradiated at 0°C suggests that a different spectrum of lesions is induced compared to Ad2 DNA gamma-irradiated at -75°C. Increased inactivation of Ad2 gamma-irradiated at -75°C when assayed on excision deficient XP25RO fibroblasts indicates that a "UV-like" DNA damage may be more abundant when gamma-irradiation occurs in the frozen state. Since both S.S. and D.S. breaks/lethal hit are not significantly higher for viral DNA gamma-irradiated at 0°C, this implies that "other" damage is responsible for the increased lethality observed when compared to DNA gamma-irradiated in the frozen state. Possible candidates for this "other" damage is base and/or sugar damage mediated by diffusable free radicals.

SUMMARY

The induction of phenotypic wild type revertants in the viral progeny from unirradiated or UV-irradiated ts early mutants of adenovirus type 5 (Ad5ts36, Ad5ts125) was examined after one cyle of lytic growth (96 hours at 33°C) in either unirradiated or UV-irradiated confluent human fibroblast strains. Reversion frequencies were scored by plaquing progeny at 33°C (permissive) or 39°C (nonpermissive) on HeLa or KB indicator cell monolayers. UV-irradiation of the virus resulted in a dose dependent increase in viral reversion for all cell strains tested. Kinetic analysis of plots of log UV-induced reversion frequency versus log UV fluence suggested that 2.5 ± 0.3 and 2.4 ± 0.5 "hits" were required to produce a reversion event in normal fibroblast strain CRL1221 for Ad5ts36 and Ad5ts125, respectively.

Following UV-irradiation of normal fibroblasts, the reversion frequency increased for both unirradiated (untargeted increase) or UV-irradiated (targeted increase) virus after an infection delay of 24 hours following cellular irradiation (10 J/m^2). The time course of expression of both UVER and UVEM responses closely paralleled the expression of the viral targeted increase such that all three parameters were maximal when viral infection was delayed 24 hours following cellular UV-irradiation. The maximal untargeted increase in normal human fibroblasts occurred when viral infection was performed immediately following cellular UV-irradiation and declined as the time course progressed. This suggested that untargeted and targeted increases may be separately regulated in normal human fibroblasts. In

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normal human fibroblast strains, UVER was found to be maximal ($3.4 \pm$ 0.8) when viral infection was delayed 24 hours following cellular UV-irradiation (10 J/m^2). Normal human fibroblasts also demonstrated a small (1.4 ± 0.2) but significant UVEM response when the host monolayers were preirradiated 24 hours prior to viral infection.

Fibroblasts from the DNA repair deficient human syndromes ataxia telangiectasia (AT), xeroderma pigmentosum (XP) and Cockayne's syndrome (CS) were also examined for viral UVER and UVEM responses. The AT fibroblast strain AT3BI when preirradiated (10 J/m^2) 24 hours prior to viral infection expressed "normal" UVER (2.5); however, UVEM values were significantly less than one (0.31). This was the result of an increased fidelity among viral progeny from UV-irradiated as compared to unirradiated AT cells infected with UV-irradiated virus. This resulted in a targeted increase significantly less than one (0.60). Untargeted increases observed for AT3BI were greater than one (1.7), similar to that observed for normal human fibroblasts infected 24 hours following cellular UV-irradiation. An abnormal UVER responses for adenovirus Vag formation was found for the immediate infection of AT strains such that the UVER for UV-irradiated Ad2 was considerably less than normal and decreased with increasing UV dose to the cells. When infection was delayed 24 hours after cellular UV-irradiation, AT strains showed apparently normal levels of UVER of UV-irradiated Ad2 using Vag information as well as plaque formation as an endpoint.

XP variant fibroblast strain CRL1162 (similar to AT3BI) expressed an increased fidelity among the viral progeny obtained from UV-irradiated (10 J/m^2) as compared to unirradiated fibroblasts

Infected with UV-irradiated virus 24 hours after cellular irradiation. This resulted in a targeted increase less than that observed for normals (0.80) whereas the untargeted increase (1.6) was similar to that observed for normals. XP variant strain CRL1162 expressed a "normal" UVER (2.1) concomitant with a UVEM (0.52) significantly less than one when viral infection was delayed 24 hours after cellular UV-irradiation. Compared to normal fibroblasts, XPA fibroblasts strain XP25RO demonstrated a UV-dose dependent hypermutability of UV-induced RF among the progeny resulting from infections of unirradiated monolayers with UV-irradiated virus. This hypermutability was not apparent if the UV-induced RF was corrected for the reduced progeny survival in XP25RO (as compared to normals) by plotting progeny RF versus lethal hits. This suggests that excision repair (deficient in XP25RO) is an error-free process in human cells. UV-irradiation of XP25RO fibroblasts (0.2 J/m^2) 24 hours prior to viral infection resulted in an untargeted increase of 2.0 for unirradiated virus and a targeted increase of 1.3 for UV-irradiated virus (200 J/m^2). Preirradiation of XP25RO therefore produced a UVEM of 0.73 and a UVER of 1.4 for UV-irradiated Ad5ts36. Thus the absence of excision repair did not allow the expression of error-prone repair processes for UV-irradiated adenovirus.

Preinfection of lethally UV-irradiated HSV-1 24 hours prior to adenovirus infection induced both targeted (3.8, 1.8) and untargeted (3.6, 1.6) increases with UVEM values of 1.1 in both GM2674 (normal) and XP25RO fibroblasts respectively. This suggests that exogenously added UV-damaged DNA may mediate mutagenesis in a transacting

mechanism.

A plot of UV-induced RF of viral progeny obtained from either unirradiated or UV-irradiated human fibroblasts (infections delayed 24 hours following cellular UV-irradiation) versus progeny lethal hits indicated large increases in RF in unirradiated fibroblasts at a damage level sufficient to produce 8-9 progeny lethal hits and in UV-irradiated fibroblasts at a damage level sufficient to produce 6-7 progeny lethal hits. This suggests that detection of UVEM using adenovirus as a probe of human cells, will be only observed at UV-damage levels sufficient to produce 6 or more lethal hits among viral progeny obtained from UV-irradiated human cells. Reduction of the number of viral lethal hits in progeny from either UV-irradiated or unirradiated cells decreased the UV-induced RF; however, a reduction in the number of lethal hits as a result of the process of UVER did not decrease the RF of reactivated viral progeny. This suggests that the process of UVER is mutagenic in human cells. Only in the cell strains AT3BI and CRL1162 is UVER accompanied by a reduction in progeny UV-induced RF suggesting that these cells may posses an inducible error-free repair mechanism that does not interfere with the UVER process. Examination of the plots of UV-induced RF versus progeny lethal hits suggests an alternate definition of UVEM that incorporates a correction for the mutagenic contribution of UVER.

Ad2 was irradiated in the liquid state at 0°C or -75°C (frozen) with gamma rays and the viral DNA was assayed for S.S. and D.S. breakage. The viral functions of adsorption and plaquing were assayed on KB cells while the HCR of Vag formation in normal and XP25RO

fibroblasts was examined and correlated with DNA breakage data. Inactivation of viral functions per unit dose was greater for virus gamma-irradiated at 0°C as compared to -75°C. The Do value for Vag formation in the excision repair deficient XP cells was (88%) of that obtained in normal human fibroblasts. Gamma-irradiation at -75°C resulted in approximately three-fold less DNA breakage per rad, a Do for plaque formation on KB cells which is more than four-fold greater, and a Do value for Vag formation in XP cells which is about 57% of that in normal fibroblasts. Thus irradiation of Ad2 at 0°C as compared to -75°C results in an alteration in the types as well as the number of viral DNA lesions.

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ABBREVIATIONS

Ad	adenovirus
AT	Ataxia telangiectasia
CS	Cockayne's Syndrome
DS	double stranded
EBV	Epstein barr virus
EDTA	ethylenediamine tetraacetic acid
EFR	error free repair
EM	enhanced mutagenesis
EMS	ethyl methane sulfonate
EPR	error-prone repair
ER	enhanced reactivation
H-1	hamster osteolytic virus
HCR	host cell reactivation
Hff	human foreskin fibroblasts
HSV	herpes simplex virus
ICdr	iododeoxycytidine
LET	linear energy transfer
MEM	minimal essential medium
MMS	methyl methane sulfonate
moi	multiplicity of infection
MR	multiplicity reactivation
PBS	phosphate buffered saline
pi	post infection
PRE	photoreactivating enzyme

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PRR	post replication repair
RF	reversion frequency
SDS	sodium dodecyl sulfate
SF	surviving fraction
SS	single stranded
SV40	simian virus 40
TBS	tris buffered saline
TK	thymidine kinase
ts	temperature sensitive
UDS	unscheduled DNA synthesis
UV	ultraviolet
UVEM	ultraviolet enhanced mutagenesis
UVER	ultraviolet enhanced reactivition
Vag	viral antigen
WM	Weigle mutagenesis
WR	Weigle reactivation
XP	Xeroderma pigmentosum