6 Nucleotide Excision-Repair Among Species

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6.1 INTRODUCTION

DNA molecules are subject to damage by a wide variety of electrophilic chemicals and by radiation. The damage can consist of alterations to the bases, the deoxyribose sugar, or the phosphodiester backbone. All organisms have evolved a series of complex interrelated enzymatic pathways whereby these different types of damage can be reversed, removed, or tolerated. This monograph addresses one relatively well-characterized pathway: the excision-repair process to remove and replace bulky damage of DNA, with special emphasis on damage produced by UV light. As an introduction, this system is described in the bacterium, *Escherichia coli*, in which the basic steps are now well understood; then the state of knowledge will be presented in detail for eukaryotic organisms, in particular yeast, *Drosophila*, and mammalian cells. The biochemistry of excision-repair *in vivo* is considered together with the properties of repair-deficient mutants, followed by the detailed understanding obtained from cloning DNA repair genes and from the characterization of gene products. A full description of this system has not yet been obtained for any eukaryotic organism. The final section of this monograph highlights interspecies similarities and differences at the levels of gene, protein, and process.

6.2 EXCISION-REPAIR IN *ESCHERICHIA COLI*

Excision-repair of UV damage in *E. coli* was first reported when wild-type cells were shown to be able to remove UV-induced pyrimidine dimers from cellular DNA, and they postulated a four-step pathway of incision, excision, repair synthesis, and resealing. At about the same time, evidence was provided for a

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non-conservative type of repair synthesis in UV-irradiated cells. Boyce and Howard-Flanders isolated mutants in three genes, *uvr A*, *uvr B*, and *uvr C*, which were shown to be deficient in excision-repair of UV damage. Subsequent work from the laboratories of Seeberg, Grossman, Rupp, Sancar and others, culminated in the cloning of the genes and isolation of the gene products involved in this excision-repair process. The enzymology of the pathway is now understood in considerable detail. References to the original work can be found in Friedberg (1985) and in the relevant chapters in Friedberg and Hanawalt (1988).

The mode of action of the *uvr ABC* enzyme is understood in great detail, although certain aspects remain controversial. The work of Orren and Sancar (1989) led to formation of a model. A dimer of the *uvr A* gene product associates with a molecule of *uvr B*, and delivers it to the damaged sites. *Uvr A* then dissociates, and *uvr C* binds to the *uvr B*–DNA complex to form the active nuclease.

The work of Grossman and coworkers (Friedberg and Hanawalt, 1988) suggested that the *uvr A* dimer binds to DNA followed by association of *uvr B*. The ternary complex is then translocated to the damaged site where the *uvr C* product associates to form the active nuclease. Irrespective of the precise mechanism, the net result is the introduction of two incisions, seven nucleotides 5′ to the pyrimidine dimer (in the case of UV damage), and three to four nucleotides 3′ to the damaged site, with a total of 12 nucleotides between the two nicks. Incisions on both sides of the damaged site by the enzyme complex had not been anticipated from *in vivo* studies, and this mechanism was revealed only following isolation and purification of the proteins. The enzyme complex remains bound on the DNA, and it is released, together with the 12-nucleotide fragment, only following the combined action of the helicase II activity encoded by the *uvr D* gene and DNA polymerase I. The latter enzyme fills in the gap left by removal of the damaged 12-nucleotide fragment, and DNA ligase joins the new stretch of DNA to the pre-existing strand.

DNA polymerase I has the ability to extend the excision gap by nick translation; but *in vitro* about 85% of the patches are 12 nucleotides long (the same size as the excised nucleotide fragment), the remaining 15% being somewhat longer. The majority of the patches *in vivo* are about 30 nucleotides in length, which may be within experimental error of the value found *in vitro*.

The *uvr ABC* enzyme has broad substrate specificity, acting primarily on damage which results in bulky distortions in DNA. These include psoralen-induced monoadducts and cross-links, as well as adducts induced by 4-nitroquinoline-1-oxide, *cis*-platinum, acetylaminofluorene, benz[*a*]pyrene-diol-epoxides, ethylating agents, higher alkylating homologs, and mitomycin C. Both principal UV-induced products—the cyclobutane pyrimidine dimer and the (6-4) photoproduct—are substrates. Recently, the enzyme complex was shown at least *in vitro* to be able to remove small lesions, such as thymine glycols and methylated bases. The physiological importance of this property of the *uvr ABC* system is unknown. The principal mechanism for removal of the latter types of DNA injury is via a completely different set of enzymes, the DNA glycosylases, which remove such

damaged bases from the DNA by rupture of the base-sugar glycosylic bonds. These enzymes are not discussed in detail in this paper.

A comparison of the *uvr ABC* enzyme with the UV-endonucleases found in T4 phage-infected *E. coli* and in *Micrococcus luteus* is instructive. The latter are small single polypeptide enzymes able to incise DNA at cyclobutane pyrimidine dimers by a different mechanism. These enzymes contain two activities, a glycosylase activity that disrupts the glycosylic bond of the 5′ pyrimidine of the dimer, and an AP endonuclease activity that breaks the phosphodiester bond between the two pyrimidines of the dimer. The net result is the breakage of the DNA backbone adjacent to 5′ of an unhooked pyrimidine dimer. The mechanism of action is thus completely different from that of the *uvr ABC* excinuclease; furthermore, these small UV-endonucleases are absolutely specific for cyclobutane pyrimidine dimers, and exert no activity on any of the other adducts listed above.

Damage to cellular DNA in *E. coli* results in the induction of the SOS response, a complex system in which at least 15 genes are induced, including both *uvr A* and *uvr B* genes whose expression increases by five- to 10-fold following UV-irradiation.

6.3 EXCISION-REPAIR IN *SACCHAROMYCES CEREVISIAE*

6.3.1 YEAST AS A MODEL SYSTEM

Currently available data support the notion that *S. cerevisiae* represents a convenient model organism for the study of excision-repair in eukaryotes, with considerable relevance for the equivalent process in mammals. As one of the most simple eukaryotes, *S. cerevisiae* has a number of important, basic characteristics that appear to be conserved throughout eukaryotic evolution. These include the main properties of chromatin structure, cell-cycle regulation, transcription, and DNA replication. This conservation is likely to extend to the principal features of DNA repair as well. On the other hand, major differences exist between yeast and mammals in aspects of mitosis, in certain types of modification of DNA (e.g., methylation), and of chromatin (e.g., poly-ADP-ribosylation), as well as in the importance and mechanisms of RNA splicing. *S. cerevisiae* genes generally have few introns.

Specific advantages of the use of this yeast species for the study of repair are:

1. its low genome complexity, being only four- to fivefold greater than that of *E. coli* (an inventory of all transcripts on the physical map of *S. cerevisiae* is expected to be completed in the near future);
2. a versatile and well studied genetic system (including haploid and diploid phases of the life cycle) which is readily amenable to powerful recombinant DNA techniques; and
3. an extensive collection of repair-deficient mutants.

The status of the knowledge on DNA repair in *S. cerevisiae* has been reviewed previously (Haynes and Kunz, 1981; Friedberg, 1988); this chapter focuses on global aspects and recent findings.

6.3.2 REPAIR MUTANTS IN *S. CEREVISIAE*

Approximately 30 different genetic loci (complementation groups) are implicated in cellular resistance to DNA damaging agents (Haynes and Kunz, 1981; Friedberg, 1988). However, the presence of a high proportion of complementation groups represented by a single mutant indicates that the *S. cerevisiae* genome is far from saturated with respect to this type of mutant (Cox and Parry, 1968). Since the more easily mutable genes are likely to be over-represented in the present mutant collection, the actual number of loci may more likely be 50 or more. Studies on the sensitivity of single and double mutants has led to the establishment of three epistasis groups: the *RAD3*, *RAD6*, and *RAD52* groups. Within one epistasis group the sensitivity of a double mutant is no higher than that of the most sensitive parent; whereas, additive or synergistic effects are found for double mutants of which the corresponding single mutants belong to different epistasis groups (Haynes and Kunz, 1981). This behavior is interpreted to mean that each epistasis group represents a distinct and non-overlapping cellular response to DNA injury: the *RAD3* epistasis group—nucleotide excision-repair; the *RAD6* group—mutagenesis and postreplication repair; and the *RAD52* group—recombination processes in response to DNA damage. Whether these groups indeed represent separate pathways and whether this subdivision will hold when knowledge expands about the genes and mutant alleles involved remain to be demonstrated.

6.3.2.1 The *RAD3* epistasis group

The *RAD3* epistasis group consists of at least 10 complementation groups all of which are sensitive to UV and in general display enhanced UV-induced mutagenesis. Five of these (*rads 1*, *2*, *3*, *4*, and *10*) are highly defective in incision of DNA-containing pyrimidine dimers or interstrand cross-links. The others (*rad 7*, *14*, *16*, *23*, and *mms 19*) exhibit a partial defect in incision of DNA after UV-exposure and an intermediate level of UV-sensitivity. Obviously, this defect could be due to leakiness of the mutant alleles. However, for *rad 7* and *rad 23*, even deletion mutations cause only a limited deficiency in repair (Schiestl and Prakash, 1989). A possible interpretation of this finding is that these mutants are involved in a subpathway of the excision-repair process. Together the *RAD3* group reveals a minimum of 10 distinct genes, participating to a greater or lesser extent in nucleotide excision-repair. In view of the likely incompleteness of the collection of yeast repair mutants, the actual number of genes implicated in this system may be even higher.

Table 6.1. Summary of sequenced *S. cerevisiae* genes induced in nucleotide excision

Gene	Chromosomal localization	Protein (predicted)	Human homolog	Remarks
RAD1	XVI	1100 aa[1]	unknown	acidic C-terminus involved in recombination
RAD2	VII	1031 aa	unknown	transcript inducible
RAD3	V	778 aa	*ERCC-2*	nucleotide, DNA binding, 5′→3′ DNA helicase, acidic C-terminus, vital function
RAD4	V	754 aa	unknown	DNA binding?[2], acidic C-terminus
RAD7	X	565 aa	unknown	acidic stretches membrane association? partial excision defect
RAD10	XIII	210 aa	*ERCC-1*	DNA binding?
CHE-3	IX	~765 aa	*ERCC-3*	nucleotide, DNA binding? acidic stretches

[1] aa = amino acids.
[2] ? = function or property postulated based on homology to known functional domains in other proteins; no direct proof at protein level.

The genes for the majority of the existing *RAD3* group mutants have been cloned (and subsequently characterized), most by transfection of yeast genomic libraries into repair deficient mutants followed by selection for UV-resistant transformants and rescue of the correcting gene (see Friedberg, 1988). The *RAD4* and *RAD7* genes were isolated based on available cloned DNA fragments containing other closely linked markers (Fleer *et al.*, 1987; Perozzi and Prakash, 1986). In addition, a yeast gene has been identified recently because of its base sequence homology with a human gene involved in excision-repair. This gene—designated *CHE-3* (for *S. cerevisiae* homologue of *ERCC-3*)—is not identical to any of the sequenced *RAD3* group genes. Table 6.1 summarizes the main features of the *S. cerevisiae* nucleotide excision-repair genes cloned and sequenced thus far (Naumovski *et al.*, 1985; Reynolds *et al.*, 1985a, b, 1987; Madura and Prakash, 1986; Perozzi and

Prakash, 1986; Gietz and Prakash, 1988; Couto and Friedberg, 1989).

The *RAD3* group genes as well as other repair genes are scattered over the yeast genome. None of the excision-repair genes contains introns. With the notable exception of *RAD2*, transcription of the *RAD3* group genes is not substantially enhanced by treatment with DNA damaging agents although a low level of induction cannot be completely excluded at this stage. This observation provides evidence in yeast against the existence of a major SOS-response mechanism that includes the excision-repair system. Furthermore, no clear evidence exists for cell cycle regulated transcription for any gene investigated for this property. Another general characteristic is the low level of expression both at the RNA and at the protein level, as suggested by the translation initiation sequence and codon usage of these genes. Apart from the apparent homologues in higher organisms, the encoded proteins have no extensive similarity to other polypeptides with a known function or involvement in other processes. As shown by Table 6.1, a high proportion of the encoded gene products harbors either acidic regions or an acidic C-terminus, either of which may confer the ability to bind chromatin as mediated by electrostatic interaction with the basic histones. Both *RAD3* and *CHE-3* proteins contain a region that perfectly matches the consensus sequence of the well-characterized nucleotide binding box found in numerous ATPases. Furthermore, *RAD3*, *RAD4*, *RAD10*, and *CHE-3* proteins may have DNA-binding domains as suggested by structural homology to "helix–turn–helix" DNA binding motifs identified in other proteins. However, only in the case of *RAD3* has ATP-dependent DNA binding been demonstrated (Sung *et al.*, 1987a). This gene was shown by Sung *et al.* (1987b) to specify a DNA-helicase capable of unwinding a double-stranded region in a DNA molecule in the 5′ to 3′ direction. In the *RAD3* protein, regions can be recognized with similarity to seven consecutive domains, found to be conserved between two superfamilies of DNA and RNA helicases (Gorbalenya *et al.*, 1989).

Gene disruption studies have disclosed that *RAD3* has a vital function in yeast as well (Higgins *et al.*, 1983; Naumovski and Friedberg, 1983), the nature of which is unknown. Of those genes examined so far, none of the other *RAD3* group genes displays such a property. However, the *RAD1* (in contrast to *rad2*, *rad3*, and *rad4*) gene appears to be involved in mitotic recombination in addition to its function in nucleotide excision (Schiestl and Prakash, 1988). Functional interactions between individual gene products of the *RAD3* group have not been demonstrated directly. Analysis of specific single and double mutants of *rad7* and *rad23* suggest, however, a functional relationship between these genes, namely, that the *RAD23* gene product can substitute for the *N*-terminal part of the *RAD7* protein (Perozzi and Prakash, 1986). Furthermore, the UV-sensitivity of double deletion mutations between *rad7* and *rad6*, and between *rad23* and *rad6* indicate a complex relationship between the *RAD7* and *RAD23* excision-repair functions and that of *RAD6* (Schiestl and Prakash, 1989). The *RAD6* gene specifies a histone 2A, 2B specific ubiquitin-conjugating enzyme (Jentsch *et al.*, 1987). Hence *RAD6* is thought to be implicated in the modulation of chromatin conformation, required for various DNA

metabolizing processes. This observation explains the extremely pleiotropic phenotype characteristic of *rad6* mutants, including defects in postreplication repair, induced mutagenesis, meiotic recombination, and sporulation (Siede, 1988). *Rad7/rad6* and *rad23/rad6* double mutants display an unexpectedly high UV-sensitivity (Schiestl and Prakash, 1989). One possible explanation for this finding is that the *RAD6* gene is also involved in a specific subpathway of excision-repair that is unaffected in the partial *rad7* and *rad23* mutants. This observation implies that *RAD6* is a gene that functions in more than one type of repair system.

6.4 *DROSOPHILA*

6.4.1 *DROSOPHILA* AS A MODEL SYSTEM

The use of the fruit fly *Drosophila melanogaster* as an experimental system offers many advantages and some disadvantages (Rubin, 1988). *Drosophila* is a multicellular highly differentiated, sexually reproducing organism, that can be subjected to detailed genetic analysis of the type to which unicellular organisms are amenable, but which are not possible with mammals. Furthermore, the *Drosophila* genome is some 15-fold smaller than that of mammals, so that, in principle, cloning of *Drosophila* genes should be correspondingly easier. The principal disadvantage is that cell culture systems of *Drosophila*, though successfully practised in several laboratories, are somewhat trickier to handle than mammalian cell culture systems, and they are not widely used on a routine basis. Nevertheless, several groups including those of Boyd and of Smith have been able to isolate many repair-deficient mutants in *Drosophila*, and to characterize the DNA repair systems in this organism. Their work has been reviewed by Boyd *et al.* (1983, 1987).

6.4.2 REPAIR-DEFICIENT MUTANTS

Repair-deficient mutants of *Drosophila* have been isolated by their hypersensitivity to mutagens, and, less directly, as mutants with increased mutation frequencies or decreased recombination. Several mutants with reduced meiotic functions (*mei* mutants) turn out to also be repair-deficient. About 30 complementation groups governing DNA repair have so far been identified. In contrast to bacterial, yeast, and mammalian systems, all the UV-sensitive *Drosophila* mutants are also sensitive to alkylating agents and in some cases to X-rays. One possible reason for this effect is that the *Drosophila* mutants were not isolated by selection for UV sensitivity, since the mutant selection procedure is carried out in whole flies, which do not easily lend themselves to UV-irradiation. Therefore, possibly a class of more specifically UV-sensitive mutants exists, and has yet to be identified.

Table 6.2. Properties of excision-repair-deficient *Drosophila* mutants

	Incision	UV endo Sites	UDS	PRR	Meiotic recombination
mei-9	0	0	---	+++	10%
mus201	0	0	---	+++	100%
mus205 100%	98	47	±		
mus302 100%	113	72	±		
mus304	90	43	±		Reduced
mus306	74	47	±		
mus308	51	24	±		

UDS = unscheduled DNA synthesis
PRR = pyrimidine dimer removal

DNA repair has been examined at the cellular level in cultured *Drosophila* cells. The steps of excision-repair, involving incision, excision, repair synthesis, and ligation are detected using similar techniques to those used for mammalian cells. The properties of some of the mutants are summarized in Table 6.2. Two mutants, *mei-9* and *mus-201*, are totally deficient in excision-repair, as measured by incision breaks, removal of pyrimidine dimers, and unscheduled DNA synthesis. In this respect, they resemble the incision-defective mutants of other species, such as the *uvr ABC* mutants of *E. coli*, the *RAD3* epistasis group of *S. cerevisiae*, at least five of the Chinese hamster complementation groups, and several of the human xeroderma pigmentosum groups. This result implies that the *mei-9* and *mus-201* mutants are defective in the incision step of excision-repair. However, in contrast to the incision-defective mutants of other species, that are only hypersensitive to UV and agents producing bulky adducts but show normal sensitivity to X-rays and alkylating agents, the *mei-9* and *mus-201* mutants of *Drosophila* are also hypersensitive to alkylating agents and, in the case of *mei-9*, to X-rays also.

Mutants at five other loci (*mus-205*, *mus-302*, *mus-304*, *mus-306*, *mus-308*) have a reduced (25 to 70%) ability to excise pyrimidine dimers, although only the *mus-306* and *mus-308* mutants are deficient in the ability to carry out the incision step following UV-irradiation. The other three mutants (*mus-205*, *mus-302*, and *mus-304*) may be defective in a later step.

6.4.3 INTERACTION OF EXCISION-REPAIR ENZYMES WITH OTHER PROCESSES

6.4.3.1 DNA replication on damaged templates

Cells from all species have the ability to tolerate damage persisting in their DNA by using an ill-characterized process termed "daughter-strand," or "postreplication repair." This process enables cells to synthesize high molecular weight intact daughter DNA strands, despite the presence of damaged sites on the parental strands. The totally excision-deficient *mei-9* and *mus-201* strains of *Drosophila* show no deficiency in this process; whereas four of the five mutants with intermediate levels of excision-repair also show a reduced level of daughter-strand repair. These observations suggest that the gene products of these loci may be involved in both repair processes.

6.4.3.2 Recombination

The central role of the *mei-9* gene product in DNA metabolism is indicated by its widely pleiotropic effects. Not only is it involved in the response to nearly all DNA damaging agents, but it also plays an important role in meiotic recombination and gene amplification. Evidence suggests that the *mei-9* product is involved in the actual meiotic exchange event. Crossing over is greatly reduced; whereas, gene conversion is unaffected. *Mei-9* mutants are also deficient in transposition and in ribosomal DNA magnification. The only other gene in any species with such a diverse series of effects is the *recA* gene in *E. coli*, which is involved in the control of the SOS response, and also plays a direct role in genetic recombination and in UV mutagenesis. In contrast to *mei-9*, however, *recA* strains of *E. coli* are relatively proficient in excision-repair of UV damage. The other totally excision-deficient *Drosophila* mutant *mus-201* shows no recombination abnormalities.

6.5 MAMMALIAN CELLS

6.5.1 CELLULAR BIOCHEMISTRY

The basic process of excision-repair of UV damage in mammalian cells is similar to that in other organisms; but the precise details of the mechanism must await the successful cloning of genes and isolation of gene products.

The cellular biochemistry of excision-repair has been studied in great detail. All steps of incision, excision, repair synthesis, and ligation can be demonstrated in cultured cells. The incision step is rate-limiting; therefore, incision breaks can be detected only either using very sensitive techniques or by blocking the subsequent repair synthesis step with inhibitors of DNA polymerases such as aphidicolin or

cytosine arabinoside (Squires *et al.*, 1982). Excision of pyrimidine dimers is effected efficiently, but fairly slowly in human cells, with most of the damage removed in about 24 hours. By contrast, cultured rodent cells are able to remove only a small fraction (10 to 30%) of pyrimidine dimers from their DNA, despite showing more or less the same resistance as human cells to the lethal effects of UV light.

A possible explanation for this paradox was provided in 1985 by the discovery that cultured hamster cells were able to remove efficiently most of the damage from actively transcribed regions of DNA, while excision from the bulk of the DNA not being actively transcribed is very inefficient (Bohr *et al.*, 1985). (This preferential repair of active genes is discussed in detail in Chapter 10 by Mullenders and Smith.) An alternative explanation for the similar sensitivity of rodent and human cells to UV-induced cell killing could be that both cell types are able to remove UV-induced (6-4) photoproducts efficiently from their DNA.

Repair synthesis, unlike replicative DNA synthesis, is not confined to the S phase of the cell cycle and occurs in all phases. This situation provides a method to measure repair synthesis termed unscheduled DNA synthesis (UDS), whereby DNA synthesis following UV-irradiation is measured autoradiographically in non-S phase cells. A further difference between repair and replicative synthesis is the relative resistance, under most conditions, of the former to the inhibitor hydroxyurea which can almost completely abolish replicative DNA synthesis. However, a combination of hydroxyurea and either cytosine arabinoside or aphidicolin can be used to block repair synthesis (Mullinger *et al.*, 1983). Since the latter is a specific inhibitor of DNA polymerase *a* and *d*, but is without effect on DNA polymerase β or γ, the latter two polymerases are not likely to be important contributors to repair synthesis; whereas one or both polymerase *a* and *d* are positively implicated. The average size of the repaired patch in human cells has been estimated to be about 30 nucleotides.

6.5.2 REPAIR-DEFICIENT MUTANTS

6.5.2.1 Human mutants

Xeroderma pigmentosum (*XP*)

The crucial importance of repair-deficient mutants in helping to dissect the DNA repair process in lower organisms is underscored by the findings described in the previous sections. The discovery by Cleaver (1968) that individuals affected with the sun-sensitive cancer-prone genetic disorder, XP were deficient in excision-repair of UV damage provided a major step toward understanding this process in human cells, and indicated the importance of excision-repair in the avoidance of cancer and in the maintenance of a healthy condition in humans. Cell fusion studies on many XP patients have enabled them to be assigned to eight distinct complementation

groups, designated A–G and variant (Lehmann, 1982a; Lehmann and Dean, 1989). Individual members originally assigned to groups H and I are now thought in fact to belong to groups D and C, respectively (Johnson *et al.*, 1989; Bootsma *et al.*, 1989). The XP variants, comprising about 20% of all XPs, are deficient in daughter strand repair, but have normal levels of excision-repair (Lehmann *et al.*, 1975). They will not be discussed further here. The other seven groups are all deficient to a greater or lesser extent in excision-repair of damage produced by UV light and by carcinogens which produce bulky adducts in cellular DNA. This deficiency results in hypersensitivity of XP cells to the lethal effects of these agents. In contrast, XP cells show a normal response to ionizing radiation, methylating agents, and other mutagens which produce only minor distortions in DNA. In this respect, they correspond to the *uvr ABC* mutants of *E. coli*, and the *RAD3* group mutants of *S. cerevisiae*.

Biochemical studies using various techniques have provided convincing evidence that all seven XP groups are deficient in an early step of excision-repair which occurs at or before the incision step (Tanaka *et al.*, 1975; De Jonge *et al.*, 1985). These findings attest to the complexity of this step in human cells. The gene products could be involved either directly in the insertion of one or more breaks near the damaged site, in preparation of the DNA structure for attack by the incision enzyme, or in altering the chromatin structure such that the damaged DNA is rendered accessible to the repair enzymes. The properties of cells in different complementation groups show some differences. Repair synthesis levels in most cells in groups A and G are extremely low; whereas in groups C and D, they are between 10 and 30% of that in normal cells (Lehmann and Dean, 1989). Nevertheless, cells in group D are very sensitive to the lethal effects of UV; whereas those in group C are less sensitive. Preliminary evidence suggests that XP-C cells are able to repair damage in active regions of DNA with some efficiency but are disturbed in the overall genome repair. In contrast, XP-D cells appear to be totally deficient in the removal of cyclobutane dimers. The residual levels of repair synthesis in XP-D strains may be caused by repair of the (6-4) photoproduct. In group F, repair synthesis is relatively slow, but it is maintained for prolonged periods (Fujiwara *et al.*, 1985).

Trichothiodystrophy (TTD)

This genetic disorder is characterized by sulphur-deficient brittle hair, mental and physical retardation, and photosensitivity in some patients. Recently, the majority of TTD patients studied were shown to have deficient excision-repair of UV damage; cell fusion studies have indicated that the defect was in the same gene as XP group D (Stefanini *et al.*, 1986). This discovery has important implications for the relationship between DNA repair and cancer (Lehmann and Norris, 1989).

Cockayne's syndrome (CS)

This genetic disorder is characterised by diverse symptoms including dwarfism, mental and physical retardation, skeletal deformities, and sun-sensitivity. Unlike those with XP, however, patients with CS show no elevated level of sunlight-induced skin cancers. Cultured CS cells are very sensitive to killing by UV light, but there no gross defect occurs in excision-repair.

Nevertheless, following UV-irradiation, RNA synthesis and DNA synthesis fail to recover to normal levels (Mayne and Lehmann, 1982). This observation led to the hypothesis—subsequently confirmed by Mayne *et al.* (1988a)—that CS cells were deficient in the ability to remove damage from active genes, while remaining proficient in excision-repair of damage from bulk DNA. The implication of this finding is that at least some of the gene products involved in preferential repair (i.e., those deficient in CS) are distinct from those involved in bulk repair. Two distinct complementation groups have been identified in CS (Lehmann, 1982b); in addition, a few patients have the symptoms of both CS and XP. One of these forms the single member of XP group B; another has been assigned to group H; recent evidence suggest that the latter cell strain may belong to group D (Johnson *et al.*, 1989).

6.5.2.2 Rodent cell mutants

The human mutants discussed above were isolated by their existence as individuals with particular clinical symptoms. Any mutant identified in this way must clearly be compatible with life in the whole individual. Therefore, mutants in other human DNA repair genes may well exist; but, they would not be compatible with life, and, therefore, could not be identified in this way.

An alternative means of obtaining mammalian cell mutants is by mutagenizing cultured mammalian cells, and selecting for mutants on the basis of their sensitivity to UV light. A major drawback to this procedure is that the mammalian genome is diploid, and the probability of obtaining a cell containing a mutation in both homologs of a particular locus is very low. Parts of the genome of cultured rodent cells are, however, functionally hemizygous, so only one copy of the genes is active in these regions. Consequently, isolation of repair-deficient rodent mutants has been possible, a process particularly useful to clone DNA repair genes (Busch *et al.*, 1989). However, that the mutant selection procedures are strongly biased in favor of mutations occurring in functionally hemizygous genes, so that some loci may not to be identified by these procedures.

Many UV-sensitive rodent cell mutants have been isolated, and is characterized genetically and biochemically. Eight complementation groups have been identified all controlling excision-repair (Zdzienicka *et al.*, 1988; Thompson *et al.*, 1988; Busch *et al.*, 1989). The properties of these mutants are summarized in Table 6.3.

Table 6.3. Properties of UV-sensitive rodent cell mutants

C-Group #	Chr #	Example	S	MMC S	T-T R	6-4 R	UDS/ incision
1	19	UV20	sss	sss	0	0	0
2	19	UV5	sss	s	0	0	0
		V79-VH-1	sss		0	50	50
3	2	UV24	sss	s	0	0	0
4	16	UV41	sss	sss	0	0	0
5	13	UV135	sss	s	0	0	0
6	10	UV61/US46	ss	s	0	100	100
7		V79-VB11	ss	s			30-70
8		L51-US31	ss	ss			

MMC = mitomycin C; UDS = unscheduled DNA synthesis; C-Group # = Complementation Group number; Chr # = Chromosome number; S = sensitivity; R = removal.

In at least five of these groups (1 to 5), the defect appears to lie in the incision step. In this respect, these mutants resemble the XP human mutants; as yet little evidence exists for overlap of mutants between the Chinese hamster and in humans. In particular, mutants in complementation groups 1 and 4 of the hamster are extremely sensitive to mitomycin C (Thompson *et al.*, 1980); whereas none of the XP groups show such sensitivity.

6.5.2.3 Repair of (6-4) photoproducts

For many years, the cyclobutane pyrimidine dimer has been recognized as a quantitatively major photoproduct produced by UV-irradiation, and its biological importance has been proven in many systems. Work carried out in recent years has shown that the (6-4) photoproduct is a minor one of some significance. Studies on mammalian cells have been carried out principally by Mitchell and coworkers, who have developed a sensitive radioimmunoassay for measuring (6-4) photoproducts (Mitchell *et al.*, 1988). Using this assay, they have shown that (a) (6-4) photoproducts are produced in cellular DNA at about 25% the frequency of cyclobutane dimers; and (b) they are removed relatively rapidly from both human and rodent cells. Most of these lesions are removed within three hours after irradiation; whereas very few cyclobutane dimers are removed during this period. The implications of these findings are that measurements of the rates of incision or

repair synthesis, which are nearly always made at early times after irradiation, will include a major contribution from the repair of (6-4) photoproducts, rather than resulting exclusively from repair of cyclobutane dimers as had previously been assumed. Thus, interpretations of some earlier work may need reevaluation.

In most of the repair-deficient mutants of both human and hamster origin, removal of both (6-4) products and cyclobutane dimers is defective (Mitchell *et al.*, 1985, 1988). However, four mutants show differences in the effect of the mutation on repair of cyclobutane dimers and on (6-4) photoproducts. The mutant VH-1 in hamster complementation group 2 is totally unable to repair cyclobutane dimers, as are other members of this group. In contrast, VH-1 is able to remove (6-4) products at about 50% of the normal rate and has a level of 50% of UDS compared with wild-type V79 cells measured during the first 3 hours after irradiation (Mitchell *et al.*, 1989). Similar properties have been found for mutant UV61 in complementation group 6 (Table 6.3). Again repair of cyclobutane dimers is absent, but repair of (6-4) photoproducts is barely affected (Thompson *et al.*, 1989). In human systems, two cell strains from patients with TTD showed opposite properties. Excision of pyrimidine dimers was normal, but repair of (6-4) photoproducts occurred at a reduced rate (Broughton *et al.*, 1990). Finally, in a revertant of a totally deficient XP-A line, repair of (6-4) products was restored; whereas repair of cyclobutane dimers in bulk DNA remained defective (Cleaver *et al.*, 1987). These findings show that, although the overall process for excision-repair of cyclobutane dimers and (6-4) products involves the same gene products, subtle differences exist perhaps in the affinity of the gene products for the two different lesions or in the domains of the proteins used in the repair of the individual lesions. Furthermore, these findings indicate that repair of both (6-4) photoproducts and of cyclobutane dimers in active genes is important for the survival of the cell.

6.5.2.4 Cloning of human repair genes

The general strategy for the isolation of mammalian repair genes is based on transfection of genomic, or cDNA, from a repair competent cell into a repair deficient mutant cell line, followed by selection of primary and secondary transformants that have nearly regained wild-type resistance. Molecular cloning of the sequences can be achieved by standard recombinant DNA techniques using tags provided by the transfected sequences themselves (species-specific dispersed repeats) or by markers linked to exogenous DNA to distinguish it from host chromosomal DNA. Presently, successful cloning of repair genes has been obtained by employing genomic DNA, although in principle full length cDNA should also be a suitable starting material. Possibly, the quality and complexity of available cDNA libraries in mammalian expression vectors were insufficiently high for the genes attempted. Because exogenous DNA is subject to scrambling in the mammalian cell during the transfection process, gene size is a limiting factor for

genomic DNA transfections. Another important parameter influencing the rate of success is the transfection and repair characteristics of a recipient cell. Different cell lines vary dramatically with respect to transfection frequencies (number of transformants per cell) as well as with regard to the average amount and degree of intactness of integrated exogenous DNA (Hoeijmakers *et al.*, 1987; Mayne *et al.*, 1988b). Unfortunately, most human cell lines (mainly SV40 transformed fibroblasts) appear to perform poorly with respect to the latter two criteria, rendering them less suitable for this approach than some rodent lines (e.g., Chinese hamster ovary, CHO) discussed below. Furthermore, the nature and stability of the repair mutation of the recipient cell line is crucially important, particularly with respect to the occurrence of revertants (Hoeijmakers *et al.*, 1988a).

The XP-A correcting (XP-AC) gene

Tanaka and coworkers (1989) succeeded in isolating the mouse and corresponding human gene that substantially and specifically enhances UV-resistance and UDS of XP-A cells. This result was obtained, notwithstanding the inferior transfection properties of the SV40 transformed XPA fibroblasts (compared with some rodent cell lines) and the occurrence of revertants noted by others (Royer-Pokora *et al.*, 1984; Schultz *et al.*, 1985).

The very extensive transfection experiments done by Tanaka *et al.* yielded two primary and one secondary transformants. The gene cloned from the secondary transformant was assigned to chromosome 9q34 (Table 6.2), in accordance with recent cell hybridization studies (e.g., Kaur and Athwal, 1989) and with a very early observation linking an XP defect with blood group markers on this chromosome (Westerveld *et al.*, 1976). The XP-AC gene was found to specify transcripts of 1.1 kb in mouse, and 1.3 kb (major) and 1.1 kb (minor) mRNA's in human cells (Tanaka *et al.*, 1989). The latter result is in good agreement with microneedle injection experiments of size-fractionated poly(A)$^+$RNA of repair competent cells into XP-A fibroblasts by Hoeijmakers *et al.* (1988b, 1990), suggesting that these two independent approaches score for the same gene. Cells of a number of XP-A patients contained either decreased amounts or abnormally sized mRNAs for this gene (Tanaka *et al.*, 1989), further strengthening the correlation between the cloned sequence and the defect in XP-A. Molecular identification of the mutation in one or more XP-A patients would provide definite proof for this proposition. A remarkable finding is that the cloned gene and cDNA correct UV-resistance and UDS only to intermediate levels (Tanaka *et al.*, 1989). This finding contrasts with complete restoration of UDS to wild type levels obtained after microinjection of partially purified XP-A correcting factor from HeLa cell extracts (De Jonge *et al.*, 1983; Vermeulen *et al.*, 1986) or calf thymus (Hoeijmakers *et al.*, 1990) when injected into XP-A fibroblasts. The XP-A correcting activity behaves as a single protein in various purification steps, binds to ss, ds, and UV-irradiated DNA with roughly the same affinity, and has a

molecular weight of 45 kD as determined by sodium dodecyl sulphate gel electrophoresis and gel filtration (Table 6.4; Hoeijmakers *et al.*, 1990). This size is sufficient to be accommodated by a 1.3 kb mRNA. However, direct proof that this protein and mRNA detected by microinjection are derived from the same cloned gene must await sequencing of the cDNA and protein.

Isolation of other XP-correcting genes

Several reports on the cloning of other XP correcting genes by DNA transfection have appeared. Unfortunately, unequivocal evidence that the cloned sequences indeed represent genes that specifically confer repair proficiency to a wild type level to representatives of the respective XP complementation groups has not been presented as yet. One of the human genes correcting a Chinese hamster repair mutant was identified as the XPB correcting gene.

The ERCC *genes*

The superior transfection properties of some rodent excision-repair mutants has permitted the cloning of several human genes by genomic DNA transfection to repair-deficient Chinese hamster lines of different complementation groups (Van Duin and Hoeijmakers, 1989). These genes are designated "*ERCC*" (for excision-repair cross complementing rodent repair deficiency) genes, followed by a number referring to the rodent complementation group. Thus the human *ERCC-1* gene complements the excision-repair defect of rodent mutants of complementation group 1. Mutants of this group are very sensitive to UV-light, carcinogens causing bulky adducts, and even more so to cross-linking agents (Thompson *et al.*, 1981; Hoy *et al.*, 1985; Zdzienicka and Simons, 1986). Transfection of the *ERCC-1* gene fully and specifically compensates for the wide spectrum of impaired repair properties of group 1 mutants (Westerveld *et al.*, 1984; Zdzienicka *et al.*, 1987; Van Duin *et al.*, 1988a). This gene is very likely not involved in any of the known XP and CS complementation groups (Van Duin *et al.*, 1989a; Table 6.4).

The *ERCC-1* gene spans a region of 15 to 17 kb on chromosome 19q13.2, and is composed of 10 exons (Van Duin *et al.*, 1987). *ERCC-1* transcripts are found at a low, basal level in all mouse tissues and in stages of embryogenesis analyzed, and do not seem to be substantially induced in UV-irradiated HeLa cells (van Duin *et al.*, 1987, 1988b). The 1.1 kb *ERCC-1* mRNA encodes a protein of 297 amino acids (Table 6.4). Comparison with consensus sequences of functional protein domains has pointed to the presence of a potential nuclear location signal (NLS) and a "helix–turn–helix" DNA binding motif (Van Duin *et al.*, 1986; Hoeijmakers *et al.*, 1986). Computer comparison of the *ERCC-1* amino acid sequence with known repair proteins of lower organisms revealed striking homology with the predicted amino acid sequence of the yeast excision-repair protein *RAD10* (van

Duin *et al.*, 1986). This finding suggests that *ERCC-1* and *RAD10* are descendants of the same ancestral gene and, hence, have analogous functions. The only major difference between the two proteins is the fact that *ERCC-1* is longer than *RAD10*. At the position where the homology with *RAD10* stops, a stretch of amino acids begins, with significant similarity with part of the *E. coli* excision-repair protein *uvrA* (Hoeijmakers *et al.*, 1986; Table 6.2). Intriguingly, at the point where this homology terminates, yet another region of similarity turns up: this time between the carboxyl terminus of *ERCC-1* and that of the *E. coli uvrC* protein (Doolittle *et al.*, 1986). Another striking similarity between the human *ERCC-1* and yeast *RAD10* genes emerged from detailed analysis of their 3′ regions. Both genes appear to overlap with the 3′ terminus of another gene (Van Duin *et al.*, 1989b). This unusual type of gene configuration seems to be conserved from yeast to humans, suggesting that it has an important biological function. At present, neither this function nor that of the antisense genes is known.

The *ERCC-2* gene cloned by Weber *et al.* (1988) corrects the incision defect and UV-sensitivity of group 2 mutants. The gene, which is approximately 20 kb in size is located on chromosome 19q13.2 in close proximity (within 250 kb) to *ERCC-1*. The cDNA sequence of ≈2.7 kb estimates a protein of 760 amino acids, with putative NLS, DNA binding, and nucleotide binding domains (Table 6.4; Weber *et al.*, 1988). The *ERCC-2* polypeptide harbors extensive homology to the yeast *RAD3* repair helicase (Weber *et al.*, 1990). Comparison of the frequency of rodent *ERCC-2* mutants generated by point mutagens versus agents causing predominantly frameshifts (which in general have a more deleterious effect) suggests that complete inactivation of *ERCC-2* (versus *ERCC-1*) is lethal and, consequently, that this gene may have a vital function in mammals (Busch *et al.*, 1989). This observation strengthens the functional homology with the *RAD3* gene in yeast.

The *ERCC-3* gene corrects the UV-sensitivity and UDS of mutants belonging to group 3 (Weeda *et al.*, 1990). The gene is ≈45 kb in size and is assigned to chromosome 2q21 (Table 6.4). The predicted 782 amino acid *ERCC-3* protein contains putative domains for nucleotide, DNA, and chromatin binding as well as for helicase activity. Its amino acid sequence bears no significant homology to known repair genes of yeast and *E. coli*. However, the gene is very strongly conserved, and recently the yeast cognate CHE-3 has been cloned in one step using human *ERCC-3* probes (Tables 6.1 and 6.4). Microinjection of the *ERCC-3* cDNA in fibroblasts of various XP complementation groups indicated that this gene specifically and completely corrects the UDS defect in XP complementation group B, the single member of which displays the clinical symptoms of both XP and CS. This finding reveals for the first time overlap between ERCC- and XP-genes.

The *ERCC-6* gene was isolated by DNA mediated gene transfer to a UV-sensitive mutant of CHO complementation group 6. The preliminary characterized gene appears to have a size of >100 kb, is located on chromosome 10q1.1, and encodes two poorly expressed mRNAs of 6.5 and 8.5 kb (Table 6.4). Determination of its nucleotide sequence should reveal whether a yeast equivalent can be recognized, and may provide hints as to its function.

Table 6.4. Summary of cloned human genes involved in nucleotide excision-repair

Gene yeast	Size properties			Chromosome location	Protein homolog
XPAC	~25 kb[1]	9q341[1]	~45 kD2[2]	Unknown	DNA binding[2]
ERCC-1	15-17 kb	19q13.2	297	*RAD10*	DNA binding?[3] Homology to parts of *uvrA*, and *uvrC*
ERCC-2	~20 kb	19q13.2	760	*RAD3*	Nucleotide, DNA binding? DNA-helicase? Vital function?
ERCC-3	~40 kb	2q21	782	*CHE-3*	Nucleotide, DNA binding? Acidic stretches
ERCC-6	~100 kb	10q1.1	>1000	Unknown	Unknown

[1]Data from gene, cloned by DNA transfection and from cell hybridization experiments.
[2]Data from partially purified protein using microinjection; formal proof that protein and gene are the same is lacking.
[3]? = property or function postulated on amino acid sequence homology to known functional domains in other proteins; direct proof at protein level lacking.

6.6 RELATIONSHIP BETWEEN REPAIR SYSTEMS OF DIFFERENT ORGANISMS

With the present state of knowledge about pro- and eukaryotic excision-repair processes, considerations are relevant of the extent to which these systems are comparable and how far extrapolation of the molecular mechanism elucidated for the *E. coli* pathway to eukaryotes is justified.

6.6.1 PROKARYOTES AND EUKARYOTES

In several respects, excision-repair is similar in pro- and eukaryotic organisms. First, the spectrum of sensitivities of excision-deficient mutants in both kingdoms is very alike, ranging from various UV-induced DNA lesions (cyclobutane dimers and (6-4) photoproducts) to bulky DNA adducts and cross-links. This variety indicates that the principal targets for both pathways overlap, and implies that mechanisms for recognition of structural alterations in DNA are likely to be strongly related. Second, several common, basic steps in the excision-repair

process have been shown to occur in both pro- and eukaryotes, although the underlying molecular mechanisms may differ markedly. This effect holds for DNA unwinding by helicases, incision of damaged DNA, repair synthesis, and ligation. However, little is known about the mechanism of incision (single or dual cut, distance from the lesion) in eukaryotes. Third, some similarity exists in the occurrence of preferential repair of the transcribed strand of DNA, found first in mammalian cells (Bohr *et al.*, 1985; Mellon *et al.*, 1987) and recently also in *E. coli* (Mellon and Hanawalt, 1989). This finding suggests that a tight linkage between the transcription process and excision-repair existed before divergence of pro- and eukaryotes and that the elements involved may have been conserved as well. Finally, at the level of the genes and proteins involved the discovery of amino acid sequence homology between part of the human *ERCC-1* and of *uvrA* and *uvrC* discussed above is of great importance. This finding implies that at least some functional aspects are shared between excision-repair proteins of *E. coli* and humans. The region of homology between *ERCC-1* and prokaryotic polypeptides, which seems to be essential for the human gene product is absent in the yeast *RAD10* protein (van Duin *et al.*, 1986). This function in yeast may not be essential, and eventually has been lost, or perhaps other yeast-repair proteins have incorporated these particular domains.

Important differences between pro- and eukaryotic excision-repair have been demonstrated, while others can be anticipated. The fundamental difference in chromatin structure between pro- and eukaryotes is expected to have major consequences for a number of DNA metabolizing cellular processes, including repair. This difference may at least explain in part the failure of *uvrABC(D)* gene products to function in intact mammalian cells (Zwetsloot *et al.*, 1986), whereas they do function in "cell-free" extracts using purified, damaged plasmid DNA as substrate (Hansson *et al.*, 1990). Another important difference, which may be related to chromatin structure, is the number of repair-deficient mutants in eukaryotes compared with those in *E. coli*. Complementation groups have been identified in yeast (10 or more) and in mammals (eight to 15), the majority of which are disturbed at incision or pre-incision steps of the excision process. This result is considerably higher than the number of genes involved in incision in *E. coli*, particularly since the values in yeast and mammals are likely to be underestimates. This observation means that the biochemical complexity, at least in the early steps of excision-repair, is probably much greater than in *E. coli*. Furthermore, except for the C-terminus of *ERCC-1*, none of the other eukaryotic excision-repair genes sequenced to date displays extensive amino acid sequence similarity to *E. coli* proteins. Finally, with respect to excision-repair itself, in none of the eukaryotic systems studied has evidence been obtained that supports the presence of a major SOS response, like in *E. coli*. The yeast RAD2 gene is the only one of the eukaryotic excision-repair genes examined thus far that is substantially induced at the transcription level by UV-irradiation.

Thus, important similarities between the molecular mechanisms of pro- and eukaryotic nucleotide excision-repair exist, perhaps in recognition of structural

aberrations in DNA and in later steps of excision-repair. At the same time, fundamental differences clearly occur, differences that originate at least in part from the principally different structure in which DNA is packaged in the two systems.

6.6.2 YEAST AND HUMANS

Available data point to a very high level of homology between the excision-repair systems of two extremes in the eukaryotic spectrum: *S. cerevisiae* and humans. Mutants from both species resemble each other in phenotype (e.g., sensitivities to various damaging agents, induced mutagenesis, lesion removal). They are also very similar with respect to the number of mutants and assignment of the defect in the majority of the mutants to (pre)incision step(s) of the excision pathway that can be bypassed by introduction of the bacteriophage T4 endonuclease (Tanaka *et al.*, 1975; De Jonge *et al.*, 1985; Valerie *et al.*, 1986). Furthermore, in high and low eukaryotes, the same basic steps in excision-repair have been demonstrated, including incision of damaged DNA, repair synthesis, ligation and preferential repair of pyrimidine dimers in actively transcribed genes. The degree of similarity is most impressive when genes are compared. All mammalian repair genes analyzed to date have a closely related cognate in yeast. This similarity concerns *ERCC-1*, with its yeast equivalent *RAD10* (*ERCC-2* which exhibits extensive amino acid sequence identity with the repair helicase *RAD3*) and *ERCC-3* (that harbors a similar level of homology with the yeast counterpart CHE-3; Tables 6.1 and 6.4). Additional genes need to be analyzed to determine whether this trend continues and whether it is representative of the entire collection of excision-repair genes. For the genes mentioned above, the similarity between the predicted gene products also implies considerable functional resemblance. Whether this degree of similarity is sufficient to permit functional cross-complementation requires thorough investigation. In the case of the couple *RAD10/ERCC-1*, expression of the *RAD10* gene in Chinese hamster mutants of group 1 resulted in a very small increase in survival following UV or MM-C treatment, which appeared independent of dose (Lambert *et al.*, 1988). This result, if significant, is difficult to rationalize with the finding that the C-terminus of the *ERCC-1* protein, which is absent in *RAD10*, is essential for its ability to confer UV and MM-C resistance to mutants of the same complementation group (Van Duin *et al.*, 1988a). Furthermore, introduction of the *ERCC-1* gene in yeast rad 10 mutants induced no significant increase in UV-survival suggesting that the *ERCC-1* protein has diverged too much from its yeast equivalent to substitute for it functionally. Clearly further research is required to see whether this observation holds also for the other combinations: *ERCC-2/RAD3*; *ERCC-3*/CHE-3 which show a considerably higher level of homology than *ERCC-1/RAD10*, and for the remaining repair genes that are and will be in the process of being cloned and analyzed.

Undoubtedly, the near future will witness considerable progress with respect to the isolation of additional genes, their evolutionary conservation, and their

functional analysis. The major challenge will be to disclose the function of each individual component, and to fit these pieces into the complex puzzle of the molecular mechanism of the excision-repair process *in vivo*.

6.6 REFERENCES

Bohr, V.A., Smith, C.A., Okumoto, D.S., and Hanawalt, P.C. (1985) DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell* **40**, 359–369.

Bootsma, D., Keijzer, W., Jung, E.G., and Bohnert, E. (1989) *Xeroderma pigmentosum* 4 complementation group XP-I withdrawn *Mutation Res.* **218**, 149–151.

Boyd, J.B., Harris, P.V., Presley, J.M., and Michael, N. (1983) *Drosophila melanogaster*: A model eukaryote for the study of DNA repair. In: *Cellular Responses to DNA Damage*, Friedberg, E.C. and Bridges, B.A. (Eds) New York: A.R. Liss, pp. 107–123.

Boyd, J.B., Mason, J.M., Yamamoto, A.H., Brodberg, R.K., Banga, S.S., and Sakaguchi, K. (1987) A genetic and molecular analyses of DNA repair in *Drosophila*. *J. Cell Sci.*, Suppl. **6**, 39–60.

Broughton, B.C., Lehmann, A.R., Harcourt, S.A., Arlett, C.F., Sarasin, A., Kleijer, W.J., Beemer, F.A., Nairn, R., and Mitchell, D.L. (1990) Relationship between pyrimidine dimers, 6-4 photoproducts, repair synthesis and cell survival: Studies using cells from patients with trichothiodystrophy. *Mutation Res.* **235**, 33–40.

Busch, D., Greiner, C., Lewis, K., Ford, R., Adair, G., and Thompson, L. (1989) Summary of complementation groups of UV-sensitive CHO cell mutants isolated by large-scale screening. *Mutagenesis* **4**, 349–354.

Cleaver, J.E. (1968) Deficiency in repair replication of DNA in *xeroderma pigmentosum*. *Nature* **218**, 652–656.

Cleaver, J.E., Cortes, F., Lutze, L.H., Morgan, W.F., Player, A.N., and Mitchell, D.L. (1987) Unique DNA repair properties of a *xeroderma pigmentosum* revertant. *Mol. Cell. Biol.* **7**, 3353–3357.

Couto, L.B., and Friedberg, E.C. (1989) Nucleotide sequence of the wild-type *RAD4* gene of *Saccharomyces cerevisiae* and characterization of mutant *rad4* alleles. *J. Bacteriol.* **171**, 1862–1869.

Cox, B.S. and Parry, J.M. (1968) The isolation, genetics and survival characteristics of ultraviolet light sensitive mutants in yeast. *Mutation Res.* **6**, 37-55.

De Jonge, A.J.R., Vermeulen, W., Klein, B., and Hoeijmakers, J.H.J. (1983) Microinjection of human cell extracts corrects xeroderma pigmentosum defect. *EMBO J.* **2**, 637–641.

De Jonge, A.J.R., Vermeulen, W., Keijzer, W., Hoeijmakers, J.H.J., and Bootsma, D. (1985) Microinjection of *Micrococcus luteus* UV-endonuclease restores UV-induced unscheduled DNA synthesis in cells of 9 xeroderma pigmentosum complementation groups. *Mutation Res.* **150**, 99–105.

Doolittle, R.F., Johnson, M.S., Husain, I., Van Houten, B., Thomas, D.C., and Sancar, A. (1986) Domainal evolution of a prokaryotic DNA repair proteins and its relationship to active-transport proteins. *Nature* **323**, 451–452.

Fleer, R., Nicolet, C.M., Pure, G.A., and Friedberg, E.C. (1987) *RAD4* gene of *Saccharomyces cerevisiae*: molecular cloning and partial characterization of a gene that is inactivated in *Escherichia coli*. *Mol. Cell Biol.* **7**, 1180–1192.

Friedberg, E.C. (1985) *DNA Repair*. Freeman: San Francisco.

Friedberg, E.C. (1988) Deoxyribonucleic acid repair in the yeast *Saccharomyces cerevisiae*. *Microbiol. Rev.* **52**, 70–102.

Friedberg, E.C. and Hanawalt, P.C. (Eds) (1988) *Mechanisms and Consequences of DNA Damage Processing*. Alan R. Liss, Inc.: New York.

Fujiwara, Y., Uehara, Y., Ichihashi, M., and Nishioka, K. (1985) Xeroderma pigmentosum 4 complementation group F: More assignments and repair characteristics. *Photochem. Photobiol.* **41**, 629–634.

Gietz, R. D. and Prakash, S. (1988) Cloning and nucleotide sequence analysis of the *Saccharomyces cerevisiae RAD4* gene required for excision-repair of UV-damaged DNA. *Gene* **74**, 535–541.

Gorbalenya, A.E., Koonin, E.V., Donchenko, A.P., and Blinov, V.M. (1989) Two related super families of putative helicases involved in replication, recombination, repair and expression of DNA and RNA genomes. *Nucleic Acids Res.* **17**, 4713-4730.

Hansson, J., Grossman, L., Lindahl, T., and Wood, R.D. (1990) Complementation of the xeroderma pigmentosum DNA repair synthesis defect with *Escherichia coli uvr ABC* proteins in a cell-free system. *Nucleic Acids Res.* **18**, 35-40.

Haynes, R.H. and Kunz, B.A. (1981) DNA repair and mutagenesis in yeast. In: *The molecular biology of the yeast* Saccharomyces. *Life cycle and inheritance*. Strathern, J., Jones, E.W., Broach, J.R. (Eds) Cold Spring Harbor Laboratory, pp. 371–414.

Higgins, D.R., Prakash, S., Reynolds, P., Polakowska, P., Weber, S., and Prakash, L. (1983) Isolation and characterization of the *RAD3* gene of *Saccharomyces cerevisiae* and inviability of *rad3* deletion mutants. *Proc. Natl Acad. Sci. USA* **80**, 5680-5684.

Hoeijmakers, J.H.J., Eker, A.P.M., Wood, R.D., and Robins, P. (1990) Use of *in vivo* and *in vitro* assays for the characterization of mammalian excision-repair and isolation of repair proteins. *Mutat. Res.* **236**, 223–238.

Hoeijmakers, J.H.J., Van Duin, M., Weeda, G., Van Der Eb, A.J., Troestra, C., Eker, A.P.M., Jaspers, N.G.J., Westerveld, A., and Bootsma, D. (1988b) Analysis of mammalian excision-repair: from mutants to genes and gene products. In: *Mechanisms and Consequences of DNA Damage Processing*. Friedberg, E.C. and Hanawalt, P.C. (Eds) Alan R. Liss Inc., pp. 281–287.

Hoeijmakers, J.H.J., Westerveld, A., and Bootsma, D. (1988a) Methods and strategies for molecular cloning of mammalian DNA repair genes by DNA-mediated gene transfer. In: *DNA repair: A Laboratory Manual of Research Procedures*. Friedberg, E.C. and Hanawalt, P.C. (Eds) Marcel Dekker Inc., pp. 181–201.

Hoeijmakers, J.H.J., Odijk, H., and Westerveld, A. (1987) Differences between rodent and human cell lines in the amount of integrated DNA after transfection. *Exp. Cell Res.* **169**, 111–119.

Hoeijmakers, J.H.J., Van Duin, M., Westerveld, A., Yasui, A., and Bootsma, D. (1986) Identification of DNA repair genes in the human genome. *Cold Spring Harbor Symp. Quant. Biol.* **51(2)**, 91–101.

Hoy, C.A., Thompson, L.H., Mooney, C.L., and Salazar, E.P. (1985) Defective DNA cross-links removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. *Cancer Res.* **45**, 1737–1743.

Jentsch, S., McGrath, J.P., and Varshavsky, A. (1987) The yeast DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme. *Nature* **329**, 131–134.

Johnson, R.T., Elliott, G.C., Squires, S., and Joysey, V.C. (1989) Lack of complementation between xeroderma pigmentosum complementation groups D and H. *Hum. Genet.* **81**, 203–210.

Kaur, G.P. and Athwal, R.S. (1989) Complementation of a DNA repair defect in xeroderma pigmentosum cells by transfer of human chromosome 9. *Proc. Natl Acad. Sci. USA* **86**, 8872–8876.

Lambert, C., Couto, L.B., Weiss, W.A., Schultz, R.A., Thompson, L.H., and Friedberg, E.C. (1988) A yeast DNA repair gene partially complements defective excision-repair in mammalion cells. *EMBO J.* **7**, 3245–3253.

Lehmann, A.R. (1982a) Xeroderma pigmentosum 4, Cockayne syndrome and ataxia-telangiectasia: Disorders relating DNA repair to carcinogenesis. In: *Cancer Surveys, 1*, Oxford University Press, pp. 93–118.

Lehmann, A.R. (1982b) Three complementation groups in Cockayne syndrome. *Mutation Res.* **106**, 347–356.

Lehmann, A.R. and Dean, S.W. (1989) Cancer prone human disorders with defects in DNA repair. In: *Handbook of Experimental Pharmacology*, Vol. 94/II, Cooper, C.S. and Grover, P.L. (Eds) Berlin: Springer-Verlag, pp. 71–101.

Lehmann, A.R., and Norris, P.G. (1989) DNA repair and cancer: speculations based on studies with xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. *Carcinogenesis* **10**, 1353–1356.

Lehmann, A.R., Kirk-Bell, S., Arlett, C.F., Paterson, M.C., Lohman, P.H.M., De Weerd-Kastelein, E.A., and Bootsma, D. (1975) Xeroderma pigmentosum cells with normal levels of excision-repair have a defect in DNA synthesis after UV-irradiation. *Proc. Natl Acad. Sci. USA* **72**, 219–223.

Madura, K. and Prakash, S. (1986) Nucleotide sequence, transcript mapping, and regulation of the *RAD2* gene of *Saccharomyces cerevisiae*. *J. Bacteriol.* **166**, 914–923.

Mayne, L.V. and Lehmann, A.R. (1982) Failure of RNA synthesis to recover after UV-irradiation: An early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. *Cancer Res.* **42**, 1473–1478.

Mayne, L.V., Mullenders, L.H.F., and Van Zeeland, A.A. (1988a) Cockayne's syndrome: A UV sensitive disorder with a defect in the repair of transcribing DNA but normal overall excision-repair. In: *Mechanisms and Consequences of DNA Damage Processing*, Friedberg, E. and Hanawalt, P. (Eds) New York: A.R. Liss, pp.349–353.

Mayne, L.V., Jones, T., Dean, S.W., Harcourt, S.A., Lowe, J.E., Priestley, A., Steingrimsdottir, H., Sykes, H., Green, M.H.L., and Lehmann, A.R. (1988b) SV40-transformed normal and DNA-repair-deficient human fibroblasts can be transfected with high frequency but retain only limited amounts of integrated DNA. *Gene* **66**, 65–76.

Mellon, I. and Hanawalt, P.C. (1989) Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature* **342**, 95–98.

Mellon, I., Spivak, G., and Hanawalt P.C. (1987) Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell* **51**, 241–249.

Mitchell, D.L., Haipek, C.A., and Clarkson, J.M. (1985) (6-4) Photoproducts are removed from the DNA of UV-irradiated mammalian cells more efficiently than cyclobutane pyrimidine dimers. *Mutation Res.* **143**, 109–112.

Mitchell, D.L., Humphrey, R.M., Adair, G.M., Thompson, L.H., and Clarkson, J.M. (1988) Repair of (6-4) photoproducts correlates with split-dose recovery in UV-irradiated normal and hypersensitive rodent cells. *Mutation Res.* **193**, 53–63.

Mitchell, D.L., Zdzienicka, M.Z., Van Zeeland, A.A., and Nairn, R.S. (1989) Intermediate (6-4) photoproduct repair in Chinese hamster V79 mutant VH-1 correlates with intermediate levels of DNA incision and repair replication. *Mutation Res.* **226**, 43–47.

Mullinger, A.M., Collins, A.R.S., and Johnson, R.T. (1983) Cell growth state determines susceptibility of repair DNA synthesis to inhibition by hydroxyurea and 1-a-d-arabinofuranosylcytosine. *Carcinogenesis* **4**, 1039–1043.

Naumovski, L. and Friedberg, E.C. (1983) A DNA repair gene required for the incision of damaged DNA is essential for viability in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **80**, 4818–4821.

Naumovski, L., Chu, G., Berg, P., and Friedberg, E.C. (1985) *RAD3* gene of *Saccharomyces cerevisiae*: nucleotide sequence of wild-type and mutant alleles, transcript mapping, and aspects of gene regulation. *Mol. Cell Biol.* **5**, 17–26.

Orren, D.K. and Sancar, A. (1989) The (A)BC excinuclease of *Escherichia coli* has only the *UvrB* and *UvrC* subunits in the incision complex. *Proc. Natl Acad. Sci. USA* **86**, 5237–5241.

Perozzi, G. and Prakash, S. (1986) *RAD7* gene of *Saccharomyces cerevisiae*: transcripts, nucleotide sequence analysis, and functional relationship between the *RAD7* and *RAD23* gene products. *Mol. Cell. Biol.* **6**, 1497–1507.

Reynolds, P., Higgins, D.R., Prakash, L., and Prakash, S. (1985a) The nucleotide sequence of the *RAD3* gene of *Saccharomyces cerevisiae*: a potential adenine nucleotide binding amino acid sequence and a nonessential carboxyl terminal domain. *Nucleic Acids Res.* **13**, 2357–2372.

Reynolds, P., Prakash, L., Dumals, D., Perozzi, G., and Prakash, S. (1985b) Nucleotide sequence of the *RAD10* gene of *Saccharomyces cerevisiae*. *EMBO. J.* **4**, 3549–3552.

Reynolds, P., Prakash, L., and Prakash, S. (1987) Nucleotide sequence and functional analysis of the *RAD1* gene of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **7**, 1012–1020.

Royer-Pokora, B. and Haseltine, W. A. (1984) Isolation of UV-resistant revertants from a xeroderma pigmentosum complementation group A cell line. *Nature* **311**, 390–392.

Rubin, G. M. (1988) *Drosophila melanogaster* as an experimental organism. *Science* **240**, 1453–1459.

Schiestl, R.H. and Prakash, S. (1988). *RAD1*, an excision-repair gene of *Saccharomyces cerevisiae*, is also involved in recombination. *Mol. Cell. Biol.* **8**, 3619–3626.

Schiestl, R.H. and Prakash, S. (1989) Interactions of the *RAD7* and *RAD23* excision-repair genes of *Saccharomyces cerevisiae* with DNA repair genes in different epistasis groups. *Curr. Genet.* **16**, 219–223.

Schultz, R.A., Barbis, D.P., and Friedberg, E.C. (1985) Studies on gene transfer and reversion to UV resistance in xeroderma pigmentosum cells. *Somatic Cell Mol. Genet.* **11**, 617–624.

Siede, W. (1988) The *RAD6* gene of yeast: a link between DNA repair, chromosome structure and protein degradation? *Radiat. Environ. Biophys.* **27**, 277–286.

Squires, S., Johnson, R.T., and Collins, A.R.S. (1982) Initial rates of DNA incision in UV-irradiated human cells: Differences between normal, xeroderma pigmentosum, and tumour cells. *Mutation Res.* **95**, 389–404.

Stefanini, M., Lagomarsini, P., Arlett, C.F., Marinoni, S., Borrone, C., Crovato, F., Trevisan, G., Cordone, G., and Nuzzo, F. (1986) Xeroderma pigmentosum 4 (complementation group D) mutation is present in patients affected by trichothiodystrophy with photosensitivity. *Hum. Genet.* **74**, 107–112.

Sung, P., Prakash, L., Weber, S., and Prakash, S. (1987a) The RAD3 gene of *Saccharomyces cerevisiae* encodes a DNA-dependent ATPase. *Proc. Natl Acad. Sci. USA* **84**, 6045–6049.

Sung, P., Prakash, L., Matson, S.W., and Prakash, S. (1987b) *RAD3* protein of *Saccharomyces cerevisiae* is a DNA helicase. *Proc. Natl Acad. Sci. USA* **84**, 8951–8955.

Tanaka, K., Sekiguchi, M., and Okada, Y. (1975) Restoration of ultraviolet-induced unscheduled DNA synthesis of xeroderma pigmentosum cells by the concomitant treatment with bacteriophage T4 endonuclease V and HVJ (Sendai virus). *Proc. Natl Acad. Sci. USA* **72**, 4071–4075.

Tanaka, K., Satokata, I., Ogita, Z., Uchida, T., and Okada, Y. (1989) Molecular cloning of a mouse DNA repair gene that complements the defect of group-A xeroderma pigmentosum. *Proc. Natl Acad. Sci. USA* **86**, 5512–5516.

Thompson, L.H., Rubin, J.S., Cleaver, J.E., Whitmore, G.F., and Brookman, K. (1980) A screening method for isolating DNA repair-deficient mutants of CHO cells. *Somatic Cell Genet.* **6**, 391–405.

Thompson, L.H., Busch, D.B., Brookman, K., Mooney, C.L., and Glaser, D.A. (1981) Genetic diversity of UV-sensitive DNA repair mutants of Chinese hamster ovary cells. *Proc. Natl Acad. Sci. USA* **78**, 3734–3737.

Thompson, L.H., Shiomi, T., Salazar, E.P., and Stewart, S.A. (1988) An eighth complementation group of rodent cells hypersensitive to ultraviolet radiation. *Somatic Cell Mol. Genet.* **14**, 605–612.

Thompson, L.H., Mitchell, D.L., Regan, J.D., Bouffler, S.D., Stewart, S.A., Carrier, W.L., Nairn, R.S., and Johnson, R.T. (1989) CHO mutant UV61 removes (6-4) photoproducts but not cyclobutane dimers. *Mutagenesis* **4**, 140–146.

Valerie, K., Fronko, G., Henderson, E.E., and De Riel, J.K. (1986) Expression of the denV gene of coliphage T4 in UV-sensitive *rad* mutants of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **6**, 3559–3562.

Van Duin, M. and Hoeijmakers J.H.J. (1989) Cloning of human repair genes by genomic DNA transfection. *Ann. Inst. Super Sanita* **25**, 131–142.

Van Duin, M., De Wit, J., Odijk, H., Westerveld, A., Yasui, A., Koken, M.H.M., Hoeijmakers, J.H.J., and Bootsma, D. (1986) Molecular characterization of the human excision-repair gene *ERCC-1*: cDNA cloning and amino acid homology with the yeast DNA repair gene *RAD10*. *Cell* **44**, 913–923.

Van Duin, M., Koken, M.H.M., Van Den Tol, J., Ten Dijke, P., Odijk, H., Westerveld, A., Bootsma, D. and Hoeijmakers, J.H.J. (1987) Genomic characterization of the human DNA excision-repair gene *ERCC-1*. *Nucleic Acids Res.* **15**, 9195–9213.

Van Duin, M., Janssen, J.H., De Wit, J., Hoeijmakers, J.H.J., Thompson, L.H., Bootsma, D., and Westerveld. A. (1988a) Transfection of the cloned human excision-repair gene *ERCC-1* to UV-sensitive CHO mutants only corrects the repair defect in complementation group-2 mutants. *Mutation Res.* **193**, 123-130.

Van Duin, M., Van Den Tol, J., Warmerdam, P., Odijk, H., Meijer, D., Westerveld, A., Bootsma, D., and Hoeijmakers, J.H.J. (1988b) Evolution and mutagenesis of the mammalian excision-repair gene *ERCC-1*. *Nucleic Acids Res.* **16**, 5305–5322.

Van Duin, M., Vredeveldt, G., Mayne, L.V., Odijk, H., Vermeulen, W., Klein, B., Weeda, G., Hoeijmakers, J.H.J., Bootsma, D., and Westerveld, A. (1989a) The cloned human DNA excision-repair gene *ERCC-1* fails to correct xeroderma pigmentosum complementation groups A through I. *Mutation Res.* **217**, 83–92.

Van Duin, M., Van Den Tol, J., Hoeijmakers, J.H., Bootsma, D., Rupp, I.P., Reynolds. P., Prakash, L., and Prakash, S. (1989b) Conserved pattern of antisense overlapping transcription in the homologous human *ERCC-1* and yeast *RAD10* DNA repair gene regions. *Mol. Cell. Biol.* **9**, 1794–1798.

Vermeulen, W., Osseweijer, P., De Jonge, A.J.R., and Hoeijmakers, J.H.J. (1986) Transient correction of excison repair defects in fibroblasts of 9 xeroderma pigmentosum

complementation groups by microinjection of crude human cell extracts. *Mutation Res.* **165**, 199–206.

Weber, C.A., Salazar, E.P., Stewart, S.A., and Thompson, L.H. (1988) Molecular cloning and biological characterization of a human gene, *ERCC2*, that corrects the nucleotide excision-repair defect in CHO UV5 cells. *Mol. Cell. Biol.* **8**, 1137–1146.

Weeda, G., Van Ham, R.C.A., Masurel, R., Westerveld, A., Odijk, H., De Wit, J., Bootsma, D., van der Eb, A.J., and Hoeijmakers, J.H.J. (1990) Molecular cloning and biological characterization of the human excision-repair gene *ERCC-3*. *Mol. Cell. Biol.*, **10**, 2570–2581.

Westerveld, A., Jongsma, A.P.M., Meera Khan, P., Van Someren, H., and Bootsma, D. (1976) Assignment of the *AK1*: *Np*: *ABO* linkage group to human chromosome 9. *Proc. Natl Acad. Sci. USA* **73**, 895–899.

Westerveld, A., Hoeijmakers, J.H.J., Van Duin, M., De Wit, J., Odijk, H., Pastink, A., Wood, R.D., and Bootsma, D. (1984) Molecular cloning of a human DNA repair gene. *Nature* **310**, 425–429.

Zdzienicka, M.Z., and Simons, J.W.I.M. (1986) Analysis of repair processes by the determination of the induction of cell killing and mutations in two repair-deficient Chinese hamster ovary cell lines. *Mutat. Res.* **166**, 59–69.

Zdzienicka, M.Z., Roza, L., Westerveld, A., Bootsma, D., and Simons, J.W.I.M. (1987) Biological and biochemical consequences of the human *ERCC-1* repair gene after transfection into a repair-deficient CHO cell line. *Mutation Res.* **183**, 69–74.

Zdzienicka, M.Z., Van Der Schans, G.P., and Simons, J.W.I.M. (1988) Identification of a new seventh complementation group of UV-sensitive mutants in Chinese hamster cells. *Mutation Res.* **194**, 165–170.

Zwetsloot, J.C.M., Barbeiro, A.P., Vermeulen, W., Arthur, H.M., Hoeijmakers, J.H.J., and Backendorf, C. (1986) Microinjection of *Escherichia coli uvrA*, *B*, *C*, and *D* proteins into fibroblasts of xeroderma pigmentosum complementation groups A and C does not result in restoration of UV-induced unscheduled DNA synthesis. *Mutation Res.* **166**, 89–98.