PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Proteins that Participate in Nucleotide Excision Repair of DNA in Mammalian Cells

Richard D. Wood

Phil. Trans. R. Soc. Lond. B 1995 **347**, 69-74
doi: 10.1098/rstb.1995.0011

Proteins that participate in nucleotide excision repair of DNA in mammalian cells

RICHARD D. WOOD

Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, U.K.

SUMMARY

The most versatile strategy for repair of damage to DNA, and the main process for repair of UV-induced damage, is nucleotide excision repair. In mammalian cells, the complete mechanism involves more than 20 polypeptides, and defects in many of these are associated with various forms of inherited disorders in humans. The syndrome xeroderma pigmentosum (XP) is associated with mutagen hypersensitivity and increased cancer frequency, and studies of the nucleotide excision repair defect in this disease have been particularly informative. Many of the XP proteins are now being characterized. XPA binds to DNA, with a preference for damaged base pairs. XPC activity is part of a protein complex with single-stranded DNA binding activity. The XPG protein is a nuclease.

1. INTRODUCTION

The main process used by cells to remove damage caused to DNA by ultraviolet light (UV) is called 'nucleotide excision repair'. It is a widely applicable repair mechanism, because in addition to removing the common UV-induced cyclobutane pyrimidine dimers and (6–4) photoproducts from irradiated DNA, a wide range of other kinds of DNA lesions can be eliminated, including many types of chemical adducts. Examples of chemical alterations acted upon by the process are adducts of purine residues with polycyclic aromatic hydrocarbons like benzpyrene diolepoxide, acetylaminofluorene linkages to guanine nucleotides, some products of reaction of cisplatin with DNA, and monoadducts and crosslinks of psoralen derivatives with pyrimidine bases. Adducts that are not removed include base pair mismatches and loopouts; these are acted upon by the separate enzymes of mismatch excision repair. Base residues modified by deamination or reaction with oxygen radicals are thought to be poor substrates for the nucleotide excision repair pathway, and these seem generally to be removed by an alternate pathway involving DNA glycosylases and apurinic endonucleases of the base excision repair pathway (see Lindahl *et al.*, this volume).

Considerable progress is now being made by many laboratories in understanding the process of nucleotide excision repair in eukaryotes, and its relationship to a fascinating group of human inherited syndromes. The importance of nucleotide excision repair is most dramatically illustrated by the existence of several human inherited syndromes in humans that are caused by mutations in repair genes. These disorders include xeroderma pigmentosum (XP), Cockayne's syndrome (CS), and trichothiodystrophy (Bootsma *et al.*, this volume). Most afflicted individuals are hypersensitive to UV light from the sun, and neurological and developmental abnormalities of various types are often found. XP patients have a greatly elevated risk of developing skin cancer in sun-exposed portions of the body.

In outline, the nucleotide excision repair mechanism in eukaryotes is reasonably simple. DNA lesions are recognized, and then two incisions occur flanking the damage to remove an oligonucleotide containing the modified residue. The oligonucleotide is excised and repair DNA synthesis takes place to form a patch. Both *in vivo* and *in vitro* measurements in human cell systems show that the repair patch formed is about 30 nucleotides long (see Shivji *et al.* 1992; Szymkowski *et al.* 1993). For UV-induced cyclobutane pyrimidine dimers and for psoralen-DNA monoadducts, an oligonucleotide of 27–32 nucleotide residues is removed, corresponding to incision $\sim$ 5 phosphodiester bonds to the 3′ side of the lesion, and $\sim$ 22–24 phosphodiester bonds to the 5′ side of the lesion (Svoboda *et al.* 1993).

From the biochemical and genetic viewpoints however, the eukaryotic mechanism is complex and more than 20 polypeptides are known to participate in the complete process (Bootsma *et al.* and Friedberg *et al.*, this volume). In mammalian cells, these proteins and their corresponding genes have been recognized in several different ways. A major class is composed of the genes and proteins that can correct the defect in XP and CS. Cell fusion studies have identified seven nucleotide excision repair-deficient complementation groups in XP (groups A to G). The XP correcting factors are required for the first steps of nucleotide excision repair, and genes or proteins for most groups have been identified within the last four years (table 1). Some XP patients are also affected with CS; these include all known XP-B patients and some in groups D and G. There are also two CS groups (CS-A and CS-B) where CS occurs independently of XP. Genes defective in these latter CS groups appear to be involved in the special situation of preferential or transcribed genes. A second category of excision repair

Phil. Trans. R. Soc. Lond. B (1995) **347**, 69–74
Printed in Great Britain

Table 1. *Some proteins involved in nucleotide excision repair in human cells*

protein	MM, from open reading frame (kDa)	apparent MM, SDS-PAGE	characteristics	other functions
XPA	31	40–42 (doublet)	Zn metalloprotein; DNA damage-binding	
XPB (ERCC3)	89	89	ATP-dependent 3′–5′ DNA helicase	component of TFIIH in human cells
XPC	106	125	binds single-stranded DNA	
HHR23B	43	58	associated with XPC	N-terminal domain has homology to ubiquitin
XPD (ERCC2)	87	80	ATP-dependent 5′–3′ DNA helicase	component of TFIIH in human cells
XPE (2 subunits)	127, ?	125, 41	binds damaged DNA	
ERCC1	33	40	part of endonuclease[a]	a pathway of mitotic recombination
XPF (ERCC4?)	?	?	part of endonuclease[a]	as above
XPG (ERCC5)	133	180–200	DNA endonuclease	
RPA (3 subunits)	68, 29, 13, 6	70, 32–34, 11–	single-stranded DNA binding	DNA replication; recombination
PCNA	29	36	formation of primer-template binding complex	DNA replication

[a] Deduced from properties of the yeast homologue; see text.

proteins is composed of the ERCC (excision repair cross-complementing) factors. These have their origin in the study of UV-sensitive mutants of rodent cell lines isolated in the laboratory. There are eleven known complementation groups of such rodent mutants, and human *ERCC* genes that can complement the repair defect in many of the groups have been isolated. There is some overlap of the ERCC, XP, and CS factors (table 1). The third category of nucleotide excision repair proteins includes those known to participate in DNA replication: RPA, PCNA, DNA polymerase ε or δ, DNA ligase, and probably other components of the DNA replication machinery.

2. SOME PROTEINS INVOLVED IN NUCLEOTIDE EXCISION REPAIR IN HUMAN CELLS

(*a*) *The XPA protein*

XPA is a 31 kDa polypeptide containing a zinc-finger motif (Miyamoto *et al.* 1992). The protein binds DNA, with a preference for damaged over undamaged duplex DNA. Preferential binding to both UV-damaged and cisplatin-damaged DNA has been demonstrated (Jones & Wood 1993). Non-damaged single-stranded DNA is also bound. In UV-irradiated DNA, (6–4) photoproducts appear to be much better substrates for binding than cyclobutane dimers, as pyrimidine dimer photolyase has little efffect on the binding of XPA to UV-irradiated DNA (Jones & Wood 1993). This preference may provide an explanation for the much higher efficiency of nucleotide excision repair in removing (6–4) photoproducts from DNA than cyclobutane pyrimidine dimers. The large difference in the rate of repair of the two lesions, known for some time *in vivo*, has been recently shown in *in vitro* systems. Normal human cell extracts repair a single (6–4) photoproduct with tenfold or greater efficiency than a cyclobutane pyrimidine dimer (Szymkowski *et al.* 1993).

The high UV-sensitivity of XP-A cell lines, the affinity of XPA for damaged DNA, and the requirement of the protein for the introduction of incisions in UV-irradiated DNA *in vivo* (Miura *et al.* 1992) and *in vitro* (Shivji *et al.* 1992) all provide strong evidence that the XPA protein is a major factor in the recognition/incision stages of DNA damage in mammalian cells. The relative affinity of XPA for a damaged versus an undamaged nucleotide pair is only several hundred to a 1000-fold (Robins *et al.* 1991; Jones & Wood 1993), and so other factors must contribute to the specificity of nucleotide excision repair in recognizing DNA damage.

(*b*) *The XPE protein*

Many research groups have found an activity in mammalian cell extracts that can bind to UV-damaged oligonucleotides in gel retardation assays. The major activity includes a polypeptide of ~ 125 kDa, distinct from XPA, that is identical with the DNA damage-binding protein first purified from human tissue by Feldberg and Grossman (1976). The binding activity has recently been co-purified with an associated 41 kDa protein by Keeney *et al.* (Keeney *et al.* 1993). Significantly, binding activity is absent in extracts from some (but not all) cell lines derived from XP-E patients (Chu & Chang 1988; Hirschfeld *et al.* 1990; Kataoka & Fujiwara 1991; Keeney *et al.* 1992). Microinjection of the two-protein complex into those XP-E fibroblasts

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

that are defective in the binding factor restores full repair synthesis to the cells (Keeney *et al.* 1994). This is a strong argument that defects in one or both of the proteins are indeed responsible for XP-E. The human gene that encodes the 125 kDa protein has been cloned (Takao *et al.* 1993). The discrimination of the XPE protein for UV photoproducts over non-damaged nucleotides is high (Hwang & Chu 1993; Reardon *et al.* 1993), being similar to the *E. coli* UvrA protein, and considerably greater than the relative affinity of XPA protein. Like XPA, the XPE protein shows a much greater affinity (on the order of tenfold) for UV-induced (6–4) photoproducts than for cyclobutane pyrimidine dimers (Abramic *et al.* 1991; Treiber *et al.* 1992; Hwang & Chu 1993; Keeney *et al.* 1993). At present, the actual role of XPE protein in repair is somewhat puzzling. XP-E cell lines are only moderately hypersensitive to UV light, and show about 50% of normal repair capability even in those cases where the DNA-damage binding activity is completely missing.

(*c*) *The ERCC1/ERCC4/XPF protein complex*

In yeast, the RAD1 and RAD10 proteins form a stable and specific complex that has endonuclease activity on single stranded DNA and can nick negatively supercoiled double stranded DNA (Sung *et al.* 1993*b*; Tomkinson *et al.* 1993, 1994). This activity is thought to be responsible for one of the specific incisions in the damaged DNA strand during nucleotide excision repair. Interestingly, the *RAD1* and *RAD10* gene products are also involved in a pathway of recombination between direct repeats in yeast, probably by removing non-homologous single-strand ends during the process (Fishman-Lobell & Haber 1992).

The human homologue of yeast RAD10 is the ERCC1 protein. *In vitro* analysis using cell-free DNA repair systems has recently revealed the existence of a complex of about 100 kDa or slightly larger that contains ERCC1, ERCC4, and XPF correcting activities (Biggerstaff *et al.* 1993; van Vuuren *et al.* 1993). By analogy with yeast, ERCC4 and XPF may be equivalent to one another and homologous to RAD1. The complex also contains an activity that corrects the repair deficiency in extracts from the sole mutant representing complementation group 11 of rodent cells (van Vuuren *et al.* 1993); the relationship of this activity to ERCC4/XPF is under investigation in several laboratories. ERCC1 and ERCC4 mutants are generally much more sensitive to chemical cross-linking agents such as mitomycin C and cisplatin than are mutants of other ERCC or XP groups (Hoy *et al.* 1985). This suggests a probable role of the complex as a nuclease in a form of DNA recombination similar to that of the RAD1–RAD10 complex in yeast.

(*d*) *The XPG protein*

During excision of a 12–13 mer oligonucleotide containing DNA damage in *E. coli*, both of the repair incisions flanking a lesion are made by the UvrB and UvrC protein complex, but two different active sites in the complex are used, one mainly involving UvrB for the 3′ incision and one involving UvrC for the 5′ incision (Lin *et al.* 1992; Lin & Sancar 1992). In eukaryotic cells the incisions are ~ 27–29 nucleotides apart and it appears likely that an even further division of labour is employed, with two different nuclease complexes used to mediate the dual incisions.

Indeed, the human XPG protein has been shown to be a further endonuclease, active on MI3 bacteriophage DNA (O'Donovan *et al.* 1994). The *S. cerevisiae* homologue of XPG is the RAD2 protein, with an overall 39% similarity confined to two domains (Scherly *et al.* 1993). Like XPG, yeast RAD2 is a nuclease (Habraken *et al.* 1993). A simplification in the list of XP and ERCC genes was made when *in vitro* analysis placed ERCC5 and XPG in the same complementation group (O'Donovan & Wood 1993). The identity of ERCC5 and XPG is confirmed by the DNA sequences of the genes (MacInnes *et al.* 1993; Scherly *et al.* 1993; Shiomi *et al.* 1994). It thus appears that two endonucleases are required during eukaryotic nucleotide excision repair, and it is likely that the incisions on the two sides of the lesion are catalysed sequentially or simultaneously by the two activities. Selective cleavage of damaged DNA must result from the interaction of these DNA endonucleases with other components of the repair complex.

(*e*) *The XPC protein complex*

Group C is one of the most common forms of XP (Kraemer *et al.* 1987). Measurements of cellular repair synthesis show that XP-C cells have 10–20% of the repair synthesis displayed by normal cells. This residual repair synthesis arises because XP-C cells repair UV-induced pyrimidine dimers in limited domains (Mansbridge & Hanawalt 1983), even though the cells are unable to remove pyrimidine dimers from most of the genome. The residual repair is strongly associated with transcriptionally active DNA (Venema *et al.* 1990), and repair of the transcribed strand of expressed genes can still take place in XP-C cells (Venema *et al.* 1991). Thus, the XPC protein appears to be involved in repair of the (non-transcribed) bulk of the genome, and may be dispensable for the repair of the transcribed strand of some or all active genes.

Recently nucleotide excision repair synthesis was reconstituted *in vitro* by combining a PCNA-depleted XP-C cell extract, PCNA and a 160 kDa XPC factor purified about 2000-fold from HeLa cells (Shivji *et al.* 1994). The XPC factor is involved in an early stage of repair, as its presence is required during the first stage of repair, before initiation of gap-filling by PCNA. However, some incisions in UV-irradiated DNA are formed by XP-C cell extracts in the absence of XPC protein. These are 'unproductive' nicks that are unstable in cell extracts and give rise to only a limited amount of synthesis in damaged DNA, most of it in open circular DNA and thus representing incomplete repair events. The XPC factor restores the ability of XP-C cell extracts to introduce incisions in damaged DNA that are stable and lead to full repair (Shivji *et al.* 1994).

Legerski and co-workers isolated a cDNA designated XPCC that corrects the DNA repair defect and UV-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

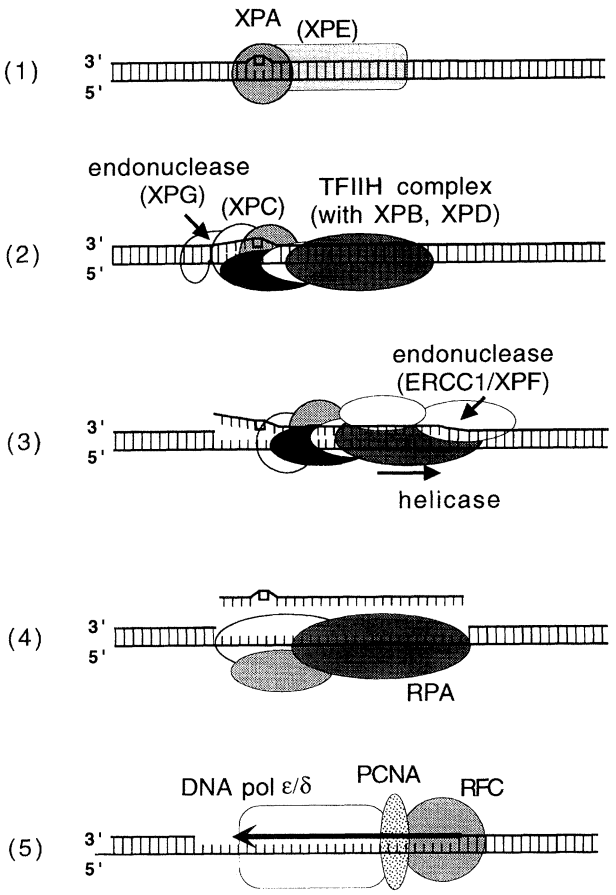

Figure 1. A speculative model for nucleotide excision repair in mammalian cells. Double-stranded DNA is indicated schematically, with the 3′ end at the left of the top strand, and damage is illustrated as a square box causing distortion of the duplex. The steps indicated from top to bottom are: (1) Recognition of a lesion in duplex DNA by XPA protein. Other proteins such as XPE may also participate in this step. (2) Incision of the damaged strand on one side of the lesion by an endonuclease. The nuclease may be correctly positioned for incision by interaction with other proteins forming a 'repairosome' that binds to DNA and promotes local denaturation around the lesion. (3) Unwinding of the damaged region by DNA helicase activities associated with TFIIH, and incision on the other side of the lesion. (4) Displacement of the damaged oligonucleotide by DNA helicase activity and single-stranded DNA binding protein. (5) Synthesis of a repair patch by a holoenzyme system consisting of DNA polymerase ε or δ, RFC, and PCNA.

The model is based on known and predicted properties of the various proteins indicated, and on the observed position of dual incisions produced by nucleotide excision repair in eukaryotes. Many features are uncertain. For instance, it is not known which nuclease complex makes the 5′ incision, and which the 3′, and in what order. Similarly, duplex unwinding near the lesion might occur before, during, or after incision.

hypersensitivity of XPC cells (Legerski & Peterson 1992). The cDNA encodes a hydrophilic polypeptide with a predicted molecular mass of 92 kDa. The cDNA shares some homology with the *S. cerevisiae* gene RAD4, but the sequence provided few clues to the function of the protein. Changes were found in this sequence in DNA from several XP-C cell lines (Li *et al.* 1993). To reconcile the 160 kDa XP-C factor that we found in HeLa cells to that encoded by the XPCC cDNA, we speculated that the XP-C factor was a protein dimer in solution, or a complex of two or more proteins. Recently, Masutani *et al.* (1994) have isolated homogeneous XP-C factor from HeLa cells as a heterodimeric complex of two proteins (XPC and HHR23B) encoded by open reading frames of 106 and 43 kDa. From their data it is apparent that the 92 kDa product identified by Legerski & Peterson (Legerski & Peterson 1992) can be formed by initiation at an ATG codon present in the 106 kDa protein, and that the true N-terminus is part of a previously undetected segment. Our hydrodynamic estimate of 160 kDa for the size of XP-C correcting activity is in reasonably good agreement with the findings of Masutani *et al.*, being intermediate between the sum of molecular masses predicted by the two open reading frames (149 kDa) and the sum of masses predicted by migration of the polypeptides on SDS-PAGE (125 + 58 = 183 kDa).

(*f*) *XPB, XPD and the TFIIH complex of proteins*

Activities that can unwind double-stranded DNA participate in many DNA metabolic processes. At least two DNA helicases are involved in the core nucleotide excision repair process in eukaryotic cells: XPD(ERCC2) and XPB(ERCC3) in human cells and their homologues RAD3 and SSL2 in yeast (see Friedberg *et al.*, this volume). These genes are of special interest because both RAD3 and SSL2 are essential for viability in yeast, a property different from the other RAD proteins. Both XPB and XPD proteins have been shown to be DNA helicases (Schaeffer *et al.* 1993; Sung *et al.* 1993*a*; Schaeffer *et al.* 1994). The DNA helicase activities of XPD and XPB may be involved in recognition of DNA damage, and/or in the displacement of the damaged oligonucleotide after incision and before repair synthesis.

Remarkably, XPB and XPD have both been found to be components of TFIIH/BTF2 (Schaeffer *et al.* 1993; Drapkin *et al.* 1994; Schaeffer *et al.* 1994; van Vuuren *et al.* 1994), a part of the RNA polymerase II basal transcription initiation complex. Functional XPB and XPD are needed for repair of non-transcribed, as well as transcribed DNA. The best interpretation of the results at present is that XPB has a dual function, serving in a DNA unwinding step during nucleotide excision repair, as well as during transcription initiation. The involvement of XPB and XPD in basal transcription may explain the clinical presentation of XP-B and XP-D patients, which includes neurological dysfunction and developmental problems in addition to the repair deficiency. For a discussion of this point the reader is referred to the article by Bootsma *et al.* in this volume.

(*g*) *DNA repair synthesis and ligation*

Nucleotide excision repair also uses proteins that function in the DNA replication machinery. RPA and PCNA proteins, previously isolated as cellular factors required for SV40 DNA replication *in vitro*, are needed for DNA repair. RPA is a trimeric single-stranded

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

DNA binding protein complex that seems to be essential for the formation and/or the stabilization of the incised intermediate structures. It may function in both early and late stages of repair (Coverley *et al.* 1991, 1992). PCNA is required for the gap-filling DNA synthesis step (Nichols & Sancar 1992; Shivji *et al.* 1992), indicating that the synthesis involves DNA polymerase δ or ε. DNA polymerase ε was purified by Nishida *et al.* (1988) as the polymerase that functioned in nucleotide excision repair in a permeabilized cell system. Recently it has been argued by M. Lee's group that DNA polymerase δ is required for nucleotide excision repair (Zeng *et al.* 1994). This conclusion is tentative, because a new method using nuclear extract protein was used to stimulate damage-dependent DNA synthesis *in vitro*, and it was not demonstrated that the synthesis was a result of nucleotide excision repair. Further study would be required to rule out the real possibility that the synthesis observed was caused by base excision repair, or even nick translation. By analogy with DNA replication, it is probable that a complex forms comprising RPA, PCNA, RFC and polymerase. To seal the nick remaining after DNA repair synthesis, three ligases are available in human cells. DNA Ligase I is a good candidate for the rejoining in nucleotide excision repair, as mutations in the corresponding gene cause a hypersensitivity to damaging agents including alkylating agents and UV (Barnes *et al.* 1992).

A speculative and schematic model of nucleotide excision repair in mammalian cells is given in figure 1, showing some possible roles for the proteins discussed here.

REFERENCES

Abramic, M., Levine, A.S. & Protić, M. 1991 Purification of an ultraviolet-inducible, damage-specific DNA-binding protein from primate cells. *J. biol. Chem.* **266**, 22493–22500.

Barnes, D.E., Tomkinson, A.E., Lehmann, A.R., Webster, A.D.B. & Lindahl, T. 1992 Mutations in the DNA ligase I gene of an individual with immunodeficiencies and cellular hypersensitivity to DNA damaging agents. *Cell* **69**, 495–503.

Biggerstaff, M., Szymkowski, D.E. & Wood, R.D. 1993 Co-correction of the ERCC1, ERCC4 and xeroderma pigmentosum group F DNA repair defects *in vitro*. *EMBO J.* **12**, 3685–3692.

Chu, G. & Chang, E. 1988 Xeroderma pigmentosum group E cells lack a nuclear factor that binds to damaged DNA. *Science, Wash.* **242**, 564–567.

Coverley, D., Kenny, M.K., Lane, D.P. & Wood, R.D. 1992 A role for the human single-stranded DNA binding protein HSSB/RPA in an early stage of nucleotide excision repair. *Nucl. Acids Res.* **20**, 3873–3880.

Coverley, D., Kenny, M.K., Munn, M., Rupp, W.D., Lane, D.P. & Wood, R.D. 1991 Requirement for the replication protein SSB in human DNA excision repair. *Nature, Lond.* **349**, 538–541.

Drapkin, R., Reardon, J.T., Ansari, A. *et al.* 1994 Dual role of TFIIH in DNA excision-repair and in transcription by RNA-polymerase-II. *Nature, Lond.* **368**, 769–772.

Feldberg, R.S. & Grossman, L. 1976 A DNA binding protein from human placenta specific for ultraviolet damaged DNA. *Biochemistry* **15**, 2402–2408.

Fishman-Lobell, J. & Haber, J.E. 1992 Removal of non-homologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene *RAD1*. *Science, Wash.* **258**, 480–484.

Habraken, Y., Sung, P., Prakash, L. & Prakash, S. 1993 Yeast excision repair gene RAD2 encodes a single-stranded DNA endonuclease. *Nature, Lond.* **366**, 365–368.

Hirschfeld, S., Levine, A.S., Ozato, K. & Protić, M. 1990 A constitutive damage-specific DNA-binding protein is synthesized at higher levels in UV-irradiated primate cells. *Molec. Cell. Biol.* **10**, 2041–2048.

Hoy, C., Thompson, L., Mooney, C. & Salazar, E. 1985 Defective DNA cross-link removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. *Cancer Res.* **45**, 1737–1743.

Hwang, B.J. & Chu, G. 1993 Purification and characterization of a human protein that binds to damaged DNA. *Biochemistry* **32**, 1657–1666.

Jones, C.J. & Wood, R.D. 1993 Preferential binding of the xeroderma pigmentosum group A complementing protein to damaged DNA. *Biochemistry* **32**, 12096–12104.

Kataoka, H. & Fujiwara, Y. 1991 UV damage-specific DNA-binding protein in xeroderma-pigmentosum complementation group E. *Biochem. biophys. Res. Commun.* **175**, 1139–1143.

Keeney, S., Chang, G.J. & Linn, S. 1993 Characterization of a human DNA-damage binding protein implicated in xeroderma pigmentosum E. *J. biol. Chem.* **268**, 21293–21300.

Keeney, S., Eker, A.P.M., Brody, T. *et al.* 1994 Correction of the DNA-repair defect in xeroderma-pigmentosum group-E by injection of a DNA damage-binding protein. *Proc. natn. Acad. Sci. U.S.A.* **91**, 4053–4056.

Keeney, S., Wein, H. & Linn, S. 1992 Biochemical heterogeneity in xeroderma-pigmentosum complementation group-E. *Mutat. Res.* **273**, 49–56.

Kraemer, K.H., Lee, M.M. & Scotto, J. 1987 Xeroderma pigmentosum: cutaneous, ocular, and neurologic abnormalities in 830 published cases. *Arch. Dermatol.* **123**, 241.

Legerski, R. & Peterson, C. 1992 Expression cloning of a human DNA-repair gene involved in xeroderma pigmentosum group C. *Nature, Lond.* **359**, 70–73.

Li, L., Bales, E.S., Peterson, C. & Legerski, R. 1993 Characterization of molecular defects in xeroderma pigmentosum group C. *Nature Genet.* **5**, 413–417.

Lin, J.J., Phillips, A.M., Hearst, J.E. & Sancar, A. 1992 Active-site of (A)BC excinuclease 2. Binding, bending, and catalysis mutants of UvrB reveal a direct role in 3′ and an indirect role in 5′ incision. *J. biol. Chem.* **267**, 17693–17700.

Lin, J.J. & Sancar, A. 1992 Active site of (A)BC excinuclease 1. Evidence for 5′ incision by UvrC through a catalytic site involving Asp399, Asp438, Asp466, and His538 residues. *J. biol. Chem.* **267**, 17688–17692.

MacInnes, M.A., Dickson, J.A., Hernandez, R.R. *et al.* 1993 Human ERCC5 cDNA-cosmid complementation for excision repair and bipartite amino acid domains conserved with RAD proteins of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Molec. Cell Biol.* **13**, 6393–6402.

Mansbridge, J. & Hanawalt, P. 1983 Domain-limited repair of DNA in ultraviolet irradiated fibroblasts from xeroderma pigmentosum complementation group C. In *Cellular responses to DNA damage* (ed. E. Friedberg and B. Bridges), pp. 195–207. New York: Alan R. Liss.

Masutani, C., Sugasawa, K., Yanagisawa, J. *et al.* 1994 Purification and cloning of a nucleotide excision repair complex involving the xeroderma pigmentosum group C protein and a human homologue of yeast RAD23. *EMBO J.* **13**, 1831–1843.

Miura, M., Domon, M., Sasaki, T., Kondo, S. & Takasaki, Y. 1992 Restoration of proliferating cell nuclear antigen

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(PCNA) complex-formation in xeroderma-pigmentosum group-A cells following cis-diammine-dichloro-platinum(II)-treatment by cell-fusion with normal-cells. *J. cell. Physiol.* **152**, 639–645.

Miyamoto, I., Miura, N., Niwa, H., Miyazaki, J. & Tanaka, K. 1992 Mutational analysis of the structure and function of the xeroderma-pigmentosum group-A complementing protein – identification of essential domains for nuclear-localization and DNA excision repair. *J. biol. Chem.* **267**, 12182–12187.

Nichols, A.F. & Sancar, A. 1992 Purification of PCNA as a nucleotide excision repair protein. *Nucl. Acids Res.* **20**, 3559–3564.

Nishida, C., Reinhard, P. & Linn, S. 1988 DNA repair synthesis in human fibroblasts requires DNA polymerase δ. *J. biol. Chem.* **263**, 501–510.

O'Donovan, A., Scherly, D., Clarkson, S.G. & Wood, R.D. 1994 Isolation of active recombinant XPG protein, a human DNA repair endonuclease. *J. biol. Chem.* **269**, 15961–15964.

O'Donovan, A. & Wood, R.D. 1993 Identical defects in DNA repair in xeroderma pigmentosum group G and rodent ERCC group 5. *Nature, Lond.* **363**, 185–188.

Reardon, J.T., Nichols, A.F., Keeney, S. *et al.* 1993 Comparative analysis of binding of human damaged DNA-binding protein (XPE) and *Escherichia coli* damage recognition protein (UvrA) to the major ultraviolet photoproducts – t[c,s]t, t[t,s]t, t[6–4]t, and t[dewar]t. *J. biol. Chem.* **268**, 21301–21308.

Robins, P., Jones, C.J., Biggerstaff, M., Lindahl, T. & Wood, R.D. 1991 Complementation of DNA repair in xeroderma pigmentosum group A cell extracts by a protein with affinity for damaged DNA. *EMBO J.* **10**, 3913–3921.

Schaeffer, L., Moncollin, V., Roy, R. *et al.* 1994 The ERCC2/DNA repair protein is associated with the class-II BTF2/TFIIH transcription factor. *EMBO J.* **13**, 2388–2392.

Schaeffer, L., Roy, R., Humbert, S. *et al.* 1993 DNA repair helicase: A component of BTF2 (TFIIH) basic transcription factor. *Science, Wash.* **260**, 58–63.

Scherly, D., Nouspikel, T., Corlet, J., Ucla, C., Bairoch, A. & Clarkson, S.G. 1993 Complementation of the DNA repair defect in xeroderma pigmentosum group G cells by a human cDNA related to yeast *RAD2*. *Nature, Lond.* **363**, 182–185.

Shiomi, T., Harada, Y.-n., Saito, T., Shiomi, N., Okuno, Y. & Yamaizumi, M. 1994 An ERCC5 gene with homology to yeast RAD2 is involved in group G xeroderma pigmentosum. *Mutat. Res.* **314**, 167–175.

Shivji, M.K.K., Eker, A.P.M. & Wood, R.D. 1994 The DNA repair defect in xeroderma pigmentosum group C and a complementing factor from HeLa cells. *J. biol. Chem.* 269, 22749–22757.

Shivji, M.K.K., Kenny, M.K. & Wood, R.D. 1992 Proliferating cell nuclear antigen is required for DNA excision repair. *Cell* **69**, 367–374.

Sung, P., Bailly, V., Weber, C., Thompson, L.H., Prakash, L. & Prakash, S. 1993*a* Human xeroderma pigmentosum group D gene encodes a DNA helicase. *Nature, Lond.* **365**, 852–855.

Sung, P., Reynolds, P., Prakash, L. & Prakash, S. 1993*b* Purification and characterization of the Saccharomyces cerevisiae RAD1–RAD10 endonuclease. *J. biol. Chem.* **268**, 26391–26399.

Svoboda, D.L., Taylor, J.S., Hearst, J.E. & Sancar, A. 1993 DNA repair by eukaryotic nucleotide excision nuclease: removal of thymine dimer and psoralen monoadduct by HeLa cell-free extract and of thymine dimer by *Xenopus laevis* oocytes. *J. biol. Chem.* **268**, 1931–1936.

Szymkowski, D.E., Lawrence, C.W. & Wood, R.D. 1993 Repair by human cell extracts of single (6–4) and cyclobutane thymine–thymine photoproducts in DNA. *Proc. natn. Acad. Sci. U.S.A.* **90**, 9823–9827.

Takao, M., Abramic, M., Moos, M. *et al.* 1993 A 127 kDa component of a UV-damaged DNA-binding complex, which is defective in some xeroderma pigmentosum group E patients, is homologous to a slime-mold protein. *Nucl. Acids Res.* **21**, 4111–4118.

Tomkinson, A.E., Bardwell, A.J., Bardwell, L., Tappe, N.J. & Friedberg, E.C. 1993 Yeast DNA repair and recombination proteins Rad1 and Rad10 constitute a single-stranded-DNA endonuclease. *Nature, Lond.* **362**, 860–862.

Tomkinson, A.E., Bardwell, A.J., Tappe, N., Ramos, W. & Friedberg, E.C. 1994 Purification of Rad1 protein from *Saccharomyces cerevisiae* and further characterization of the Rad1/Rad10 endonuclease complex. *Biochemistry* **33**, 5305–5311.

Treiber, D.K., Chen, Z.H. & Essigmann, J.M. 1992 An ultraviolet light-damaged DNA recognition protein absent in xeroderma pigmentosum group E cells binds selectively to pyrimidine (6–4) pyrimidone photoproducts. *Nucl. Acids Res.* **20**, 5805–5810.

van Vuuren, A.J., Appeldoorn, E., Odijk, H., Yasui, A., Jaspers, N.G.J. & Hoeijmakers, J.H.J. 1993 Evidence for a repair enzyme complex involving ERCC1, ERCC4, ERCC11 and the xeroderma pigmentosum group F proteins. *EMBO J.* **12**, 3693–3701.

van Vuuren, A.J., Vermeulen, W., Weeda, G. *et al.* 1994 Correction of xeroderma pigmentosum repair defect by basal transcription factor BTF2 (TFIIH). *EMBO J.* **13**, 1645–1653.

Venema, J., van Hoffen, A., Karcagi, V., Natarajan, A.T., van Zeeland, A.A. & Mullenders, L.H.F. 1991 Xeroderma pigmentosum complementation group-C cells remove pyrimidine dimers selectively from the transcribed strand of active genes. *Molec. Cell Biol.* **11**, 4128–4134.

Venema, J., van Hoffen, A., Natarajan, A.T., Van Zeeland, A.A. & Mullenders, L.H.F. 1990 The residual repair capacity of xeroderma pigmentosum complementation group C fibroblasts is highly specific for transcriptionally active DNA. *Nucl. Acids Res.* **18**, 443–448.

Zeng, X.R., Jiang, Y.Q., Zhang, S.J., Hao, H.L. & Lee, M.Y.W.T. 1994 DNA-polymerase-delta is involved in the cellular-response to UV damage in human-cells. *J. biol. Chem.* **269**, 13748–13751.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES