DNA repair in human cells: from genetic complementation to isolation of genes

D BOOTSMA, A WESTERVELD and J H J HOEIJMAKERS
Department of Cell Biology and Genetics, Erasmus University, Rotterdam

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Keywords: DNA repair, cancer, xeroderma pigmentosum, complementation, gene cloning, evolutionary gene conservation.

Summary

The genetic disease xeroderma pigmentosum (XP) demonstrates the association between defective repair of DNA lesions and cancer. Complementation analysis performed on XP cell strains and on repair deficient rodent cell lines has revealed that at least nine and possibly more than 13 genes are involved in early steps of the excision of ultraviolet light-induced DNA lesions in mammalian cells. Two of these genes have been cloned and others are in an advanced stage of cloning. One cloned gene, *ERCC–1*, has been characterized at the molecular level. This human gene is homologous with excision repair genes in yeast and in *Escherichia coli*. These results indicate that the excision repair system is conserved during evolution. It is expected that the cloning and characterization of prokaryotic and eukaryotic repair genes will pave the way to a deeper understanding of mammalian repair systems and their association with cancer.

I Living cells repair damaged DNA

The integrity of the genetic code in living cells is threatened by alterations due to DNA damaging agents in the environment, instability of certain chemical bonds in DNA and mistakes in the complex DNA replication process. The cells answer these threats by the action of DNA repair systems which prevent lesions from interfering with essential cellular functions. A complex network of repair systems has evolved to cope with different types of DNA damage

(see Friedberg, 1985, for review). Most of our understanding of these processes is based on studies of DNA repair systems in *Escherichia coli* by analysis of radiation-sensitive mutants. Cloning of the genes and purification of the corresponding proteins have elicited two repair systems in particular: the excision repair pathway of ultraviolet light-induced DNA lesions and bulky adducts (for review see Sancar, 1987) and the adaptive response directed towards alkylation lesions (Teo *et al*, 1986). The excision repair pathway which is part of the SOS response in *E coli* is depicted in Fig. 1. The

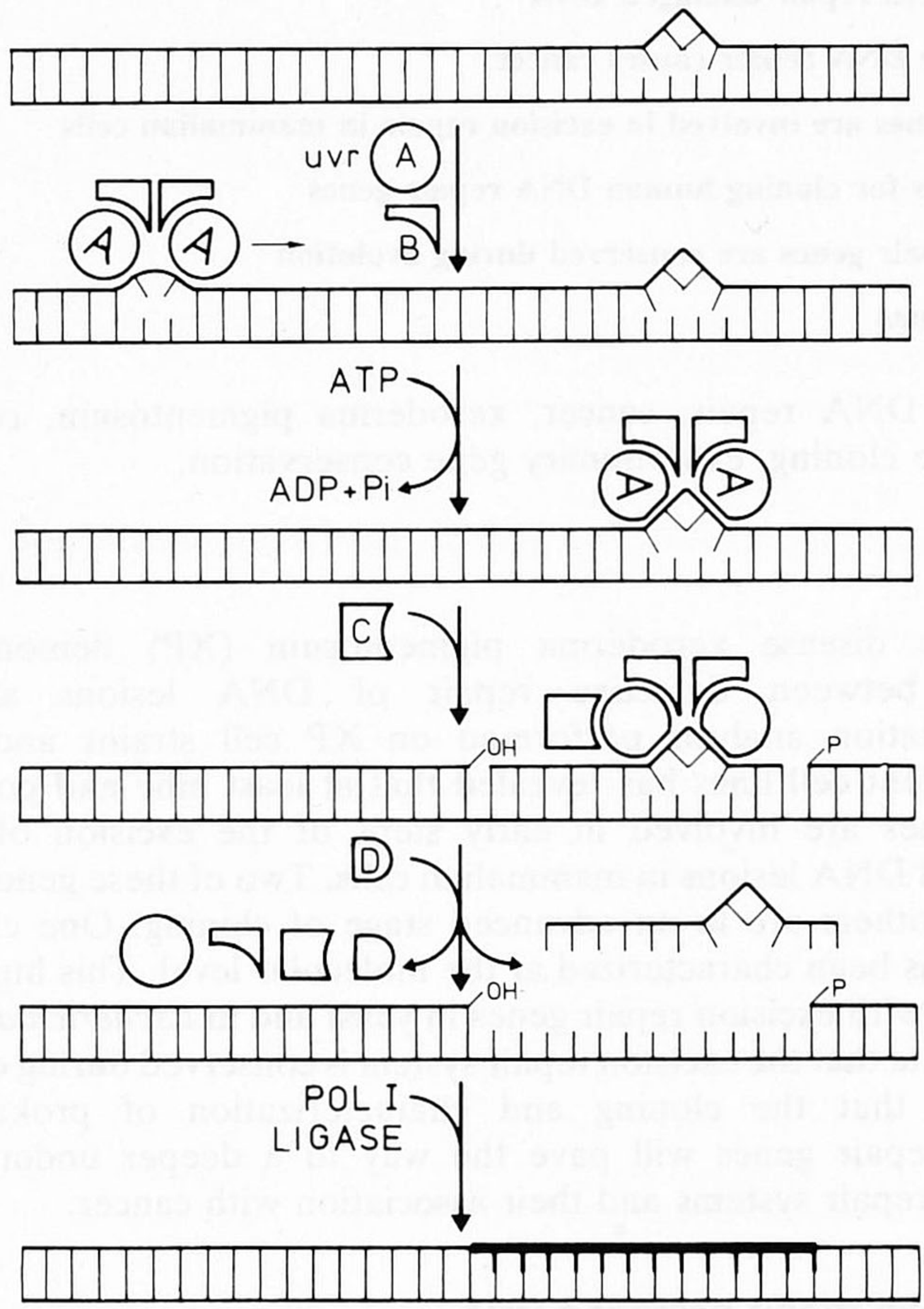

Fig. 1. Model for excision repair in *Escherichia coli*. The uvr A protein binds to the DNA and in the presence of uvr B a ternary (presumably dimeric) complex is formed which displays partial unwinding activity, consumes ATP and translocates along the DNA until it encounters a lesion (eg a pyrimidine dimer). The complex pauses at sites with altered DNA conformation for which it has a high affinity. The uvr C protein is required to initiate a dual incision event in the damaged DNA strand: the 8th phosphodiester band 5′ and the 4th or 5th phosphodiester band 3′ to the DNA injury are hydrolysed. Dissociation of the complex and displacement of the 12–13 nucleotide oligomer is achieved by the combined action of uvr D (helicase II) and DNA polymerase I. Finally, the excision gap is filled in by the polymerase and sealed by ligase

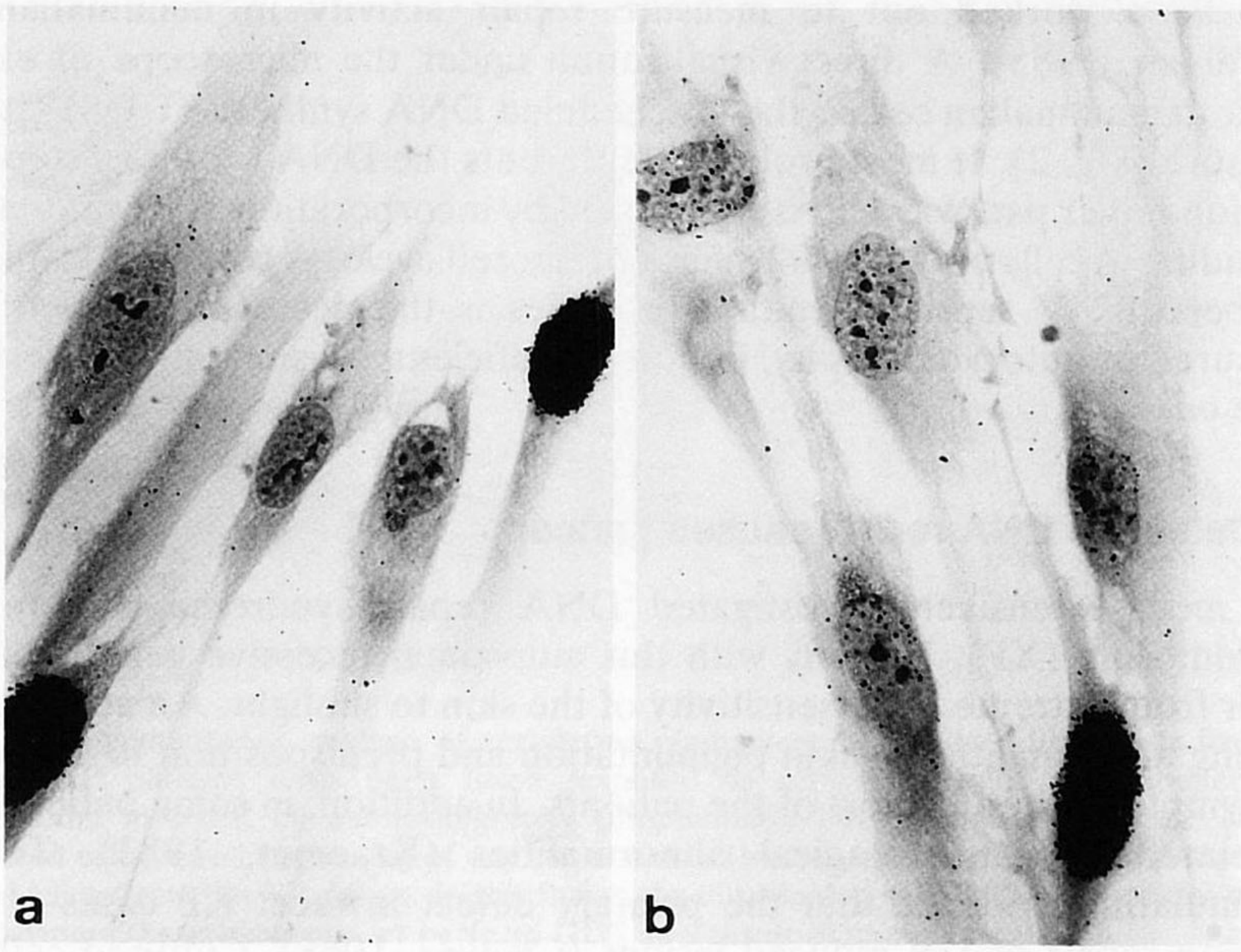

Fig. 2. Ultraviolet light-induced DNA repair synthesis in human fibroblasts. (a) Unexposed culture showing two cells in S-phase (labelled with ^{3}H–thymidine followed by autoradiography). Cells in G1 or G2-phase are unlabelled. (b) Cells exposed to 10 J/m^2 UV. Cells in G1 and G2-phase are weakly labelled as a result of UV-induced unscheduled DNA synthesis

products of at least four different genes, uvr A, uvr B, uvr C and uvr D, act together to remove the DNA lesions. Uvr A binds to the DNA and in conjunction with uvr B a stable complex is formed that by partial unwinding of the DNA helix (Oh and Grossman, 1987) controls the integrity of the genetic code and is blocked at the site of a lesion. Incision of the damaged strand, which occurs at both sides of the lesions, is catalysed in the presence of the uvr C protein. The unwinding activity of the uvr D gene product stimulates the release of the 12–13 nucleotide fragment containing the lesion. Finally, DNA synthesis and ligation complete the excision repair process.

Evidence is accumulating that homologous repair systems are operational in eukaryotic cells. In *Saccharomyces cerevisiae* at least 10 loci have been identified which are involved in the genetic control of excision repair of UV-induced DNA lesions (based on genetic analysis of radiation sensitive, RAD, yeast mutants). The rapid progress in cloning of the genes followed by analysis of their gene products will contribute extensively to understanding the functioning of these genes.

As far as mammalian cells are concerned valuable tools for the study of repair and the identification of repair genes are mutant, repair deficient cell lines. These are derived from two sources: (a) by isolation from established (mainly rodent) cell lines of mutant cells sensitive to DNA damaging agents and (b) by culturing cells from the skin or other tissues of patients showing hypersensitivity to DNA damaging agents. A large number of test systems

have been worked out to measure repair activity in mammalian cells (Friedberg, 1985). A direct visualization under the microscope of excision repair in mammalian cells is the unscheduled DNA synthesis (UDS) after UV exposure (Fig. 2). It most probably represents the DNA synthesis step in the excision repair pathway and is manifested by incorporation of tritium-labelled thymidine in cells in G1 or G2-phase of the cell cycle. A common feature of a number of UV sensitive mutant cell lines is the decreased level of UDS measured by autoradiography, indicating deficient excision of DNA lesions in these cells.

II Defective DNA repair causes cancer

The most extensively investigated DNA repair syndrome is xeroderma pigmentosum (XP). Patients with this autosomal recessive genetic disorder suffer from extreme hypersensitivity of the skin to sunlight. An atrophic skin, striking abnormalities in skin pigmentation and predisposition to skin cancer are symptoms seen in most of the patients. In addition, in some patients XP is associated with neurological abnormalities (Kraemer, 1983). There is accumulating evidence that the primary defect in most XP cases is in the excision repair of UV-induced lesions (Cleaver, 1968). Cultured cells from XP patients are extremely sensitive to UV exposure and show significantly reduced levels of UDS. Despite 20 years of extensive research in laboratories all over the world the precise biochemical nature of the defect(s) in XP cells has not yet been elucidated. A small group of XP patients (XP variant) seems to be deficient in a process termed 'postreplication repair'. The nature of that repair system is not well understood; experimental evidence suggests that the defect in XP variant cells interferes with the replication of damaged DNA.

Recently, in another cancer prone genetic disease, Bloom's syndrome (BS), a defective ligase I activity was demonstrated (Chan *et al*, 1987; Willis and Lindahl, 1987). This disease is characterized by stunted growth and light-induced capillary dilatation of the skin of the face in typical butterfly distribution. BS cells in culture show a high incidence of spontaneous chromosome aberrations and sister chromatid exchanges (Kraemer, 1983).

Two other chromosome breakage syndromes, ataxia telangiectasia and Fanconi's anaemia, also characterized by predisposition to cancer, may be the result of DNA repair deficiencies as well (Kraemer, 1983). However, direct evidence supporting this assumption is lacking. At the present time XP and BS present the most convincing illustration of the association of cancer with defective repair of DNA damage.

III Many genes are involved in excision repair in mammalian cells

Genetic studies performed by fusion of cells from different XP patients have shown the existence of at least nine complementation groups within the excision deficient class of XP patients (de Weerd-Kastelein *et al*, 1972; Fischer *et al*, 1985). This complementation analysis is based on the determination of UDS in multinucleated cells in which the genetic

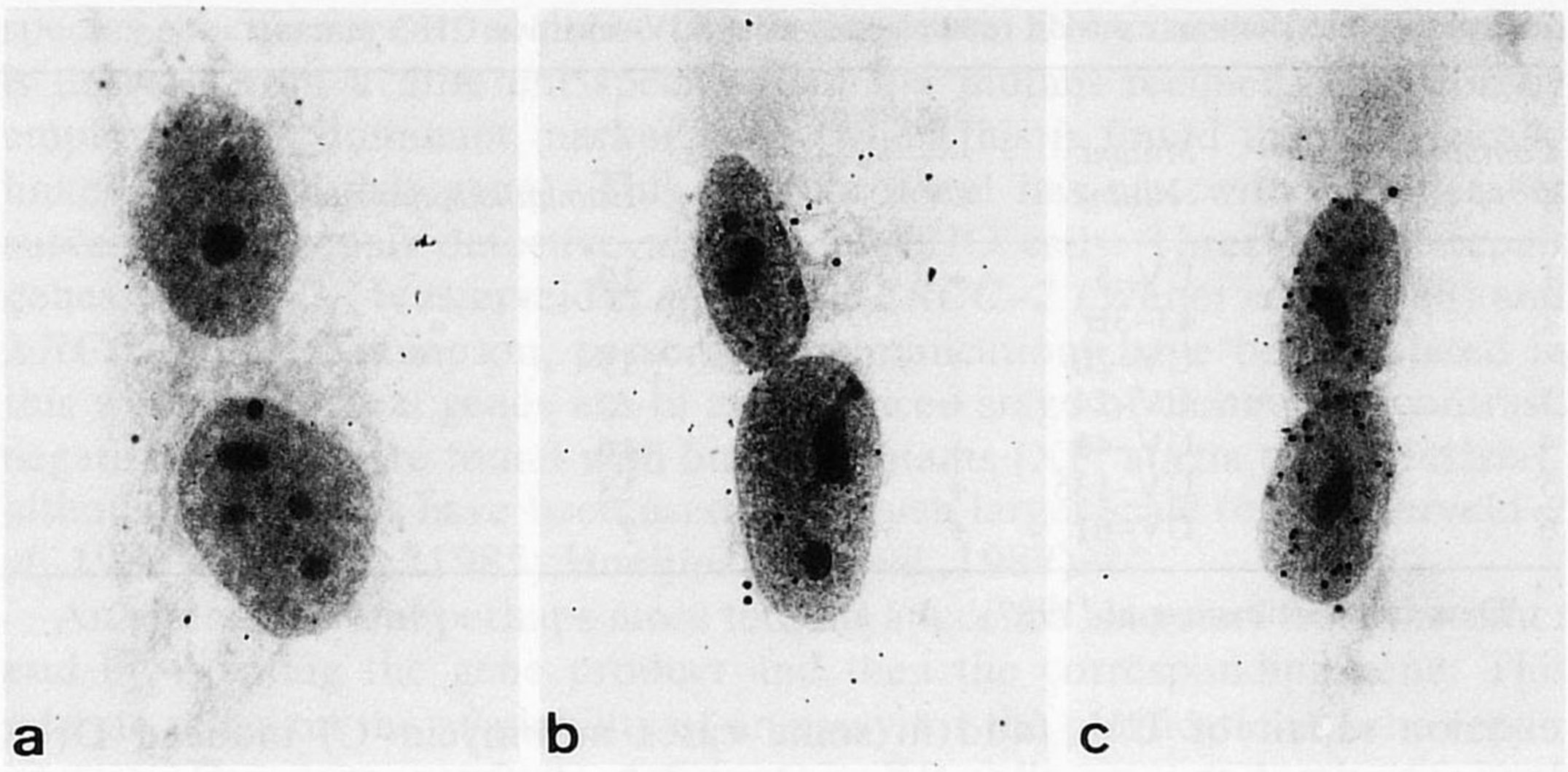

Fig. 3. Complementation analysis of xeroderma pigmentosum. Cultured fibroblasts from two unrelated XP patients have been fused, exposed to ultraviolet light and labelled with ^{3}H–thymidine followed by autoradiography (unscheduled DNA synthesis test, see Fig. 2). Binucleated cells containing nuclei of one patient only (homokaryons, a and b) do not perform UDS, which reflects the XP defect. In this fusion the binucleated cells containing nuclei of both fusion partners (heterokaryons, c) perform UDS as a result of complementation

information of two XP patients is combined (Fig. 3). Restoration of UDS up to the level of wild-type cells is taken as evidence for the presence of mutations in different genes in the fusion partners (intergenic complementation, though intragenic complementation cannot be excluded). Biochemical and cell biology studies have indicated that the defects in the nine XP complementation groups reside in early steps of an excision repair pathway, probably before or at the incision step.

Complementation analysis of excision deficient CHO mutant cell lines, performed by Thompson and coworkers (Thompson *et al*, 1981, 1987; Thompson and Carrano, 1983), has revealed the presence of six complementation groups. At least five of these groups are, like XP, unable to perform efficiently the first step postulated in the excision pathway, the incision of the damaged DNA strand at or near the photolesion (Thompson *et al*, 1987). It is not known whether some of the complementation groups are the same in both species. So far a limited number of complementation tests by fusion of XP cells with CHO mutants have been performed (Stefanini *et al*, 1985; Thompson *et al*, 1985). The hybrids tested to date have shown complementation, indicating that the human and Chinese hamster mutations examined are in different genes. In proliferating hybrids obtained after fusion of CHO mutants with normal human cells segregation of the repair proficient phenotype with human chromosomes is observed. This suggests that human cells possess the genes which are mutated in the Chinese hamster cells. Chromosome analysis of these hybrids allowed the assignment of these genes to specific human chromosomes (Table; Siciliano *et al*, 1987). These complementation and assignment data suggest the participation of at least nine and possibly more than 13 genes and proteins in the early steps of

Identification of human excision repair genes using UV-sensitive CHO mutants

Complementation group	*Mutant cell line*	*Sensitivity to:*		*Human chromosome*[1]
		UV	*MM–C*	
1	UV–5	+	–	19
2	43–3B			
	UV–20	+	+	19
3	UV–24	+	–	2
4	UV–41	+	+	16
5	UV–135	+	–	13
6	UV–61	+	–	?

[1]Data from Siciliano *et al* (1987)

excision repair of UV- (and in some cases mitomycin–C) induced DNA lesions.

IV Strategies for cloning human DNA repair genes

Isolation of the mammalian DNA repair genes discussed above can provide the means to analyse molecular mechanisms of repair systems. Gene structure, expression and regulation are directly amendable to study. Elucidation of the nucleotide sequence yields important information on the encoded gene product. Computer comparison of the deduced amino acid sequence with that of other proteins can pinpoint regions of homology, reveal evolutionary relationships and—when complemented with site directed mutagenesis—can identify functional domains. Finally, the cloned gene can be utilized for the overproduction of the corresponding protein in *E coli* as a prelude to the characterization of the polypeptide and its role in the repair process. A recent example of the power of this approach is the detailed insight that is gained into the molecular intricacies of the excision repair system of *E coli* (Sancar and Rupp, 1983).

A variety of strategies can be adopted to isolate mammalian DNA repair genes. One of the most straightforward approaches is based on 'correction' of repair deficient mutants by DNA-mediated gene transfer of the normal gene present in the genome of repair competent cells, using the calcium phosphate DNA coprecipitation technique (Graham and van der Eb, 1973). Transfectants that have incorporated the correcting gene are selected from the transformed mutant population by treatment with the appropriate mutagen (eg UV). The selection step is considerably facilitated when plasmid vectors, containing dominant marker genes, are added to the chromosomal DNA. These genes specify products that enable host cells to survive selection with a specific agent (eg antibiotic). This provides a means of preselecting the small fraction of transfection-competent recipient cells (generally less than 0.1 % of the transfected cell population). Furthermore, dominant markers can be used to 'tag' the transfected genomic DNA and the repair gene. DNA of repair proficient transformants is used to construct a recombinant DNA library in *E coli*. Clones harbouring the repair gene can be identified by using

species specific repeats as probe (when the donor DNA used for transfection is derived from a different species than the mutant recipient cells) or by employing the dominant marker gene (when this is found to be physically linked to the repair gene). This basic protocol has met with considerable success using repair defective mutants of CHO cells. Three human repair genes, *ERCC–1* (Westerveld *et al*, 1984), *ERCC–2* (Weber *et al*, 1988) and *XRCC–1* (LH Thompson, personal communication) have been isolated in this way and several genes are in an advanced stage of cloning. In contrast, negative results were found with human mutants (XP, ataxia telangiectasia), although these cells have been used on a much larger scale (eg Westerveld *et al*, 1984; Lehmann, 1985; Hoeijmakers *et al*, 1987).

An alternative but perhaps more tedious approach is to start from the other end by isolating the gene product and then the corresponding gene. This scheme relies on the availability of an assay for the purification of the repair protein. For gene products deficient in XP cells we—and others—have developed an in vivo assay system based on transient correction of XP excision defects by the normal proteins present in crude extracts of repair proficient cells (de Jonge *et al*, 1983; Yamaizumi *et al*, 1986). The missing component is introduced into the cytoplasm of XP fibroblasts by microneedle injection and temporary correction is measured at the single cell level by determining the rate of UV-induced UDS. In this way specific alleviation of the defect was achieved in all nine excision deficient XP complementation groups after injection of normal or heterologous XP extracts but not after the introduction of homologous extracts (Vermeulen *et al*, 1986). The protein correcting the XP–A deficiency was found to precipitate at between 25% and 40% $(NH_4)_2SO_4$ saturation, to be retained on phosphocellulose, heparin sepharose and various dye matrix columns and to exhibit a high affinity for UV irradiated double-stranded DNA as well as single-stranded DNA. We are currently purifying this factor from calf thymus on a large scale. Two main routes can be followed from the protein to the gene. The first approach involves determination of part of the amino acid sequence (eg from the NH_2-terminus) followed by synthesis of an oligonucleotide (mixture) deduced on the basis of the degeneration of the code from a suitable portion of the amino acid sequence. This oligonucleotide mixture can subsequently be used as a probe to screen either a cDNA or a genomic DNA library for hybridizing colonies. The second strategy uses antibodies raised against the purified protein. The antibodies can be used to screen a bacterial cDNA expression library. Obviously many other strategies may be envisaged to investigate human repair genes and it is useful that these are being explored in different laboratories. It is, however, beyond the scope of this paper to discuss all of these extensively. Possible strategies are described by Friedberg *et al* (1987).

V DNA repair genes are conserved during evolution

ERCC–1 is the first human DNA repair gene to be cloned and its characterization has proceeded the furthest (van Duin *et al*, 1986). This gene

fully complements the excision repair defect of CHO mutants belonging to CHO complementation group 2. Mutants of this group are extremely vulnerable to UV light and agents causing bulky DNA adducts but are also vulnerable to crosslinking agents (Table). None of the other four excision defective CHO complementation groups is corrected by *ERCC–1*. Investigations into whether one of the XP complementation groups is disturbed in the *ERCC–1* function have yielded negative results. Transfection of the *ERCC–1* gene to SV40 transformed fibroblasts representative of XP complementation groups A, C, D, E and F and an immortalized Fanconi's anaemia cell strain (group A) did not result in correction of the repair deficiency. Furthermore, no gross rearrangements or large deletions have been detected in the *ERCC–1* gene on Southern blots of genomic DNA from cell lines of all nine XP groups (unpublished results). The *ERCC–1* gene is located on chromosome 19 band q13.2. Accidently, the two other repair genes isolated, *ERCC–2*, correcting excision defective mutants of CHO group 1 (Weber *et al*, 1988), and the *XRCC–1* gene, complementing the *x* ray sensitivity of CHO mutant EM–9 (LH Thompson, personal communication), have been assigned to the same chromosome (Siciliano *et al*, 1986). Molecular analysis of *ERCC–1* has revealed that the gene spans a region of 15-17 kb. It is composed of 10 exons one of which (exon VIII, 72 bp) appears to be alternatively spliced. The differential RNA processing yields two mRNAs of 1.1 and 1.0 kb (Fig. 4, corresponding cDNAs pcDE and pcDE–72 respectively). Minor longer transcripts of 3.4 and 3.8 kb have been detected as well (Fig. 4, pcDE + Xb). These arise by the use of alternative polyadenylation sites (unpublished observations). The various *ERCC–1* transcripts encode largely identical polypeptides of 297 and 273 amino acids, depending on the presence or absence of exon VIII (Fig. 5). Transfection of the two cDNAs (pcDE and pcDE–72) inserted in mammalian expression vectors has shown that the repair defect of complementation group 2 mutant cells can be corrected solely by the longer transcript (van Duin *et al*, 1986).

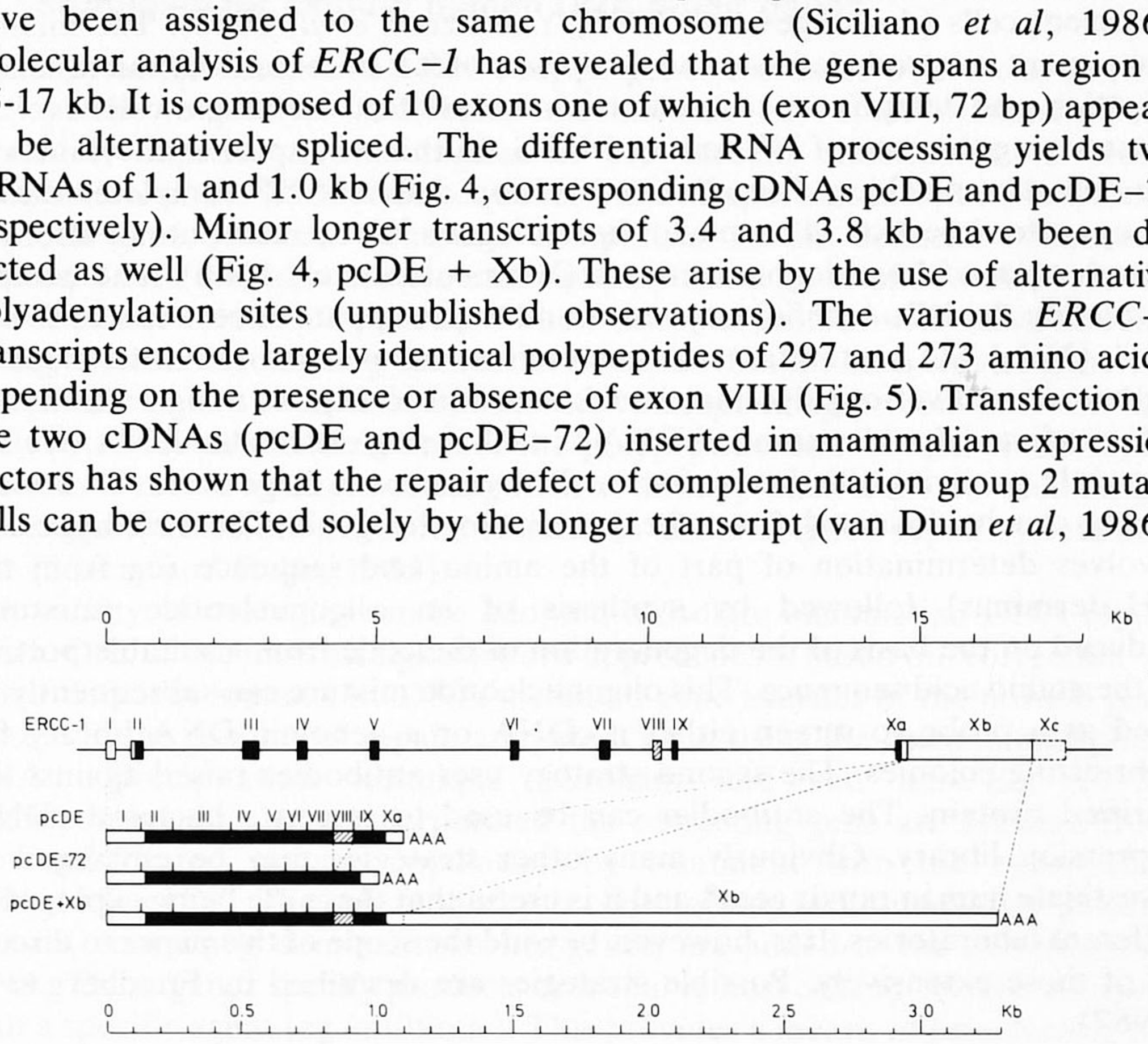

Fig. 4. Schematic representation of the structure of the *ERCC–1* gene and various cDNAs. The exons (indicated by roman numerals) and introns of the *ERCC–1* gene are drawn to scale. Three *ERCC–1* cDNAs are shown in the bottom half of the figure. Coding regions are indicated by the filled boxes. The alternatively spliced exon VIII, which is also coding, is shown by the shaded box. Note that the scales for gene and cDNAs are different.

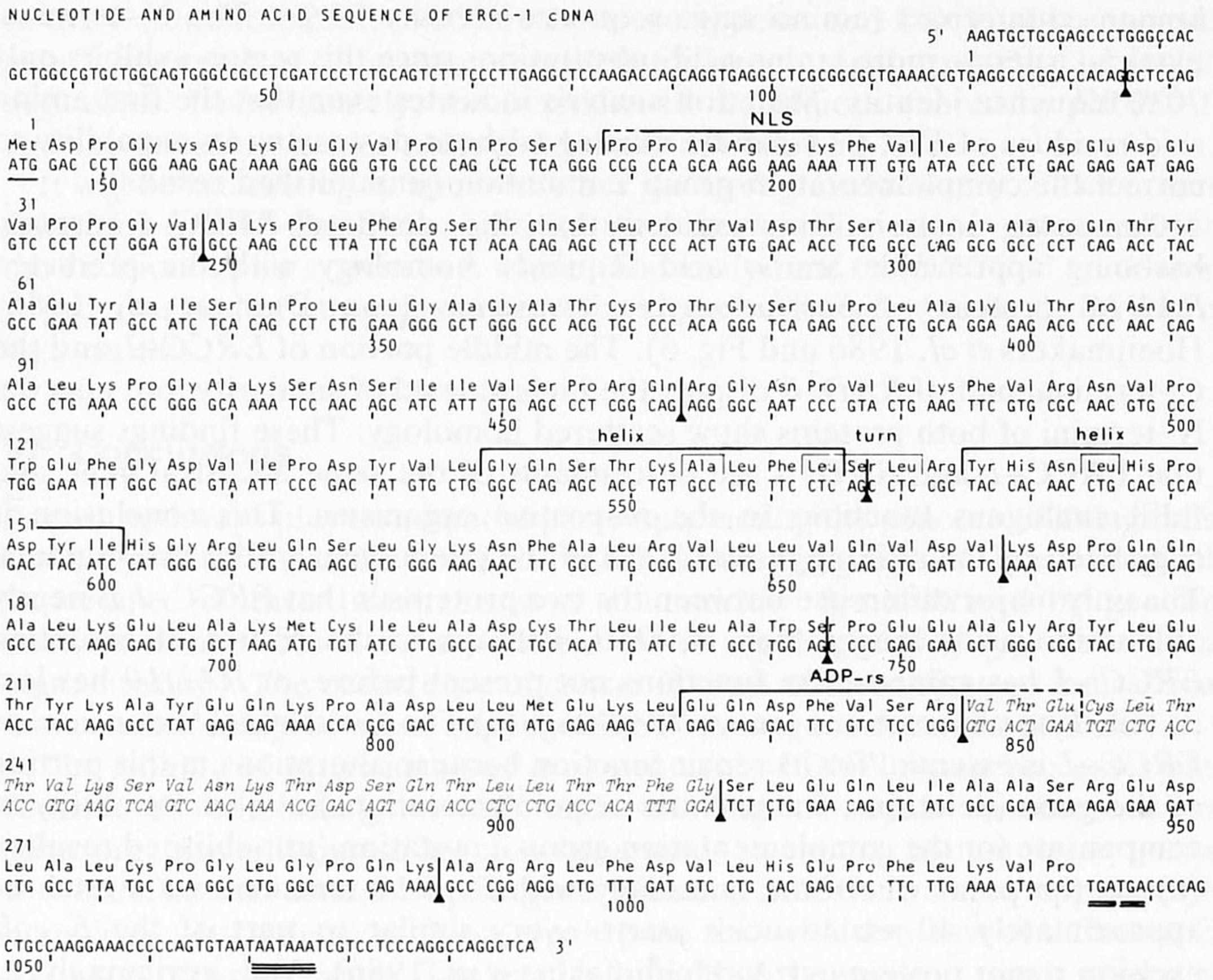

Fig. 5. Nucleotide and deduced amino acid sequence of *ERCC–1* cDNA clone pcDE. The position of exon borders is indicated by ▲. The alternatively spliced exon VIII (72 bp) absent in pcDE–72 is printed in italics. Potential functional domains: NLS = nuclear location signal; helix-turn-helix = DNA binding domain; ADPrs = ADP monoribosylation site. The stop codons and polyadenylation signal AATAAA used for pcDE and pcDE–72 are underlined

The biological importance and function of the smaller mRNA (pcDE–72) is still obscure.

Concerning the *ERCC–1* gene product comparison with consensus sequences of functional protein domains has pointed to some potential functions hidden in the *ERCC–1* amino acid sequence (Hoeijmakers *et al*, 1986 and Fig. 5): (a) A region that strongly resembles the well characterized nuclear location signal of the SV40 T antigen. (b) A part showing structural homology with the 'helix-turn-helix' motive implicated in DNA binding and identified in a number of prokaryotic and eukaryotic DNA binding proteins. (c) A possible ADP-monoribosylation site only present in the large *ERCC–1* gene product. Definite proof for these functional domains awaits verification at the protein level.

The *ERCC–1* gene is strongly conserved. In ZOO-blot analysis specific hybridization is observed with DNAs of all vertebrates tested (including reptiles and fish) and even with *Drosophila*. Moreover, the nucleotide sequence of the cloned mouse *ERCC–1* gene shows that particularly the last two thirds of the gene and protein are very similar to the

human counterpart (amino acids sequence identity 93%). The N–terminal part can tolerate more amino acid substitutions since this region exhibits only 70% sequence identity. Mutation analysis indicates even that the first amino acid residues of *ERCC–1* can be missing without destroying its capability to correct the complementation group 2 mutation (unpublished results).

Computer analysis has revealed that the deduced *ERCC–1* protein harbours appreciable amino acid sequence homology with the predicted *RAD10* protein of *Saccharomyces cerevisiae* (van Duin *et al*, 1986; Hoeijmakers *et al*, 1986 and Fig. 6). The middle portion of *ERCC–1* and the C–terminal half of *RAD10* display the highest level of similarity, but also the N–termini of both proteins show scattered homology. These findings suggest that *ERCC–1* and *RAD10* are descendants of the same ancestral gene and fulfil analogous functions in the respective organisms. This conclusion is supported by the striking resemblance of the phenotypes of the two mutants. The only major difference between the two proteins is that *ERCC–1* is nearly 100 amino acids longer than *RAD10*. Perhaps in the course of evolution *ERCC–1* has gained extra functions not present before, or *RAD10* has lost its tail. Two observations are worth noting: (a) The C–terminal 'extension' of *ERCC–1* is essential for its repair function because alterations in this portion of the gene (deletions, frame-shifts or point mutations) abolish its ability to compensate for the complementation group 2 mutation (unpublished results). (b) At the point where the homology with *RAD10* terminates a stretch of approximately 40 amino acids starts—very similar to part of the *E coli* excision repair protein uvr A (Hoeijmakers *et al*, 1986). And, intriguingly, at the position where this homology stops again another region of identity turns up: this time between the 60 C–terminal amino acids of *ERCC–1* and those

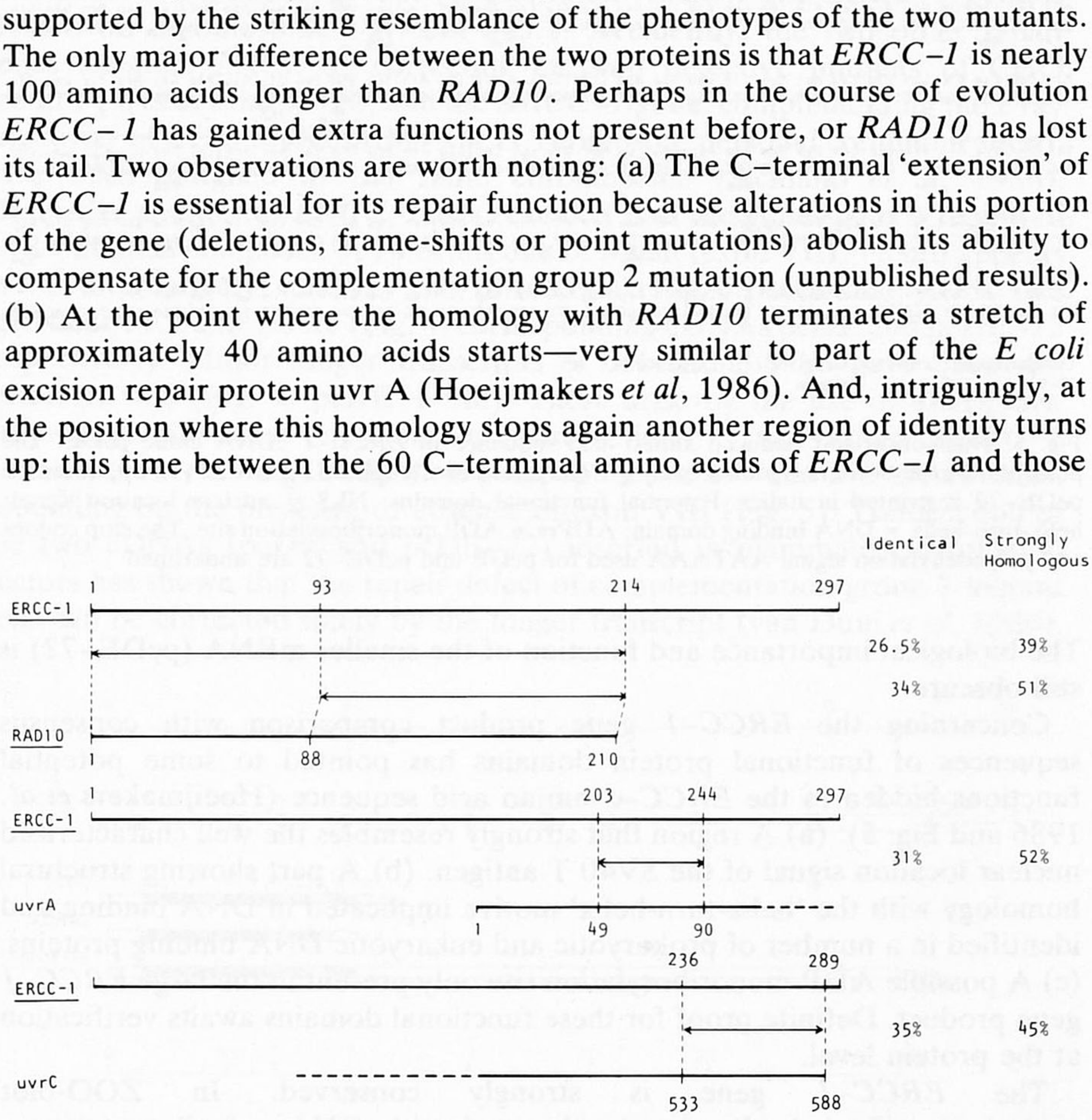

Fig. 6. Schematic representation of homology between *ERCC–1* protein and the *RAD10*, uvrA and uvrC proteins. The table on the right gives percentages of homology of the regions indicated by arrows between the dashed lines. The numbering corresponds to the amino acid sequence of the proteins

of uvr C (Doolittle *et al*, 1986). It therefore appears that *ERCC–1* is a mosaic gene composed of domains present in different repair proteins of lower organisms. Unfortunately, the functions of the regions shared by *ERCC–1* and the microbial repair polypeptides are not known.

It will be interesting to know whether the strong evolutionary conservation found for *ERCC–1* will also hold for the other excision repair genes and for the excision repair system as a whole. The analysis of the *ERCC–2* gene now in progress and of other genes yet to be cloned will undoubtedly throw more light on this.

VI Conclusions

Repair pathways play an essential part in protecting the genome of living cells against genetic alterations caused by DNA damaging agents. The biological importance of these mechanisms is dramatically demonstrated by genetic diseases in man based on a deficient DNA repair pathway (eg xeroderma pigmentosum). The occurrence of cancer in most of these diseases indicates a direct relationship between repair of DNA damage and carcinogenesis.

The cloning of genes involved in the control of DNA repair processes has contributed extensively to the understanding of repair mechanisms in microorganisms. Progress has now been made in cloning human repair genes as well. The first human repair gene isolated, *ERCC–1*, shows appreciable homology with a yeast excision repair gene and with domains of different *E coli* excision repair genes. The results imply that the excision repair system is strongly conserved in evolution and opens up the possibility of isolating human genes by virtue of their homology with excision repair genes available from yeast.

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Dedication

This paper is dedicated to Professor Guido Pontecorvo on his eightieth birthday

References

Chan JYH, Becker FF, German J and Ray JH (1987) Altered DNA ligase I activity in Bloom's syndrome cells. *Nature* **325** 357–359

Cleaver JE (1968) Defective repair replication of DNA in xeroderma pigmentosum. *Nature* **218** 652–656

Doolittle RF, Johnson MS, Husain I, van Houten B, Thomas DC and Sancar A (1986) Domainal evolution of a prokaryotic DNA repair protein and its relationship to active-transport proteins. *Nature* **323** 451–453

Duin van M, de Wit J, Odijk H, Westerveld A, Yasui A, Koken MHM, Hoeijmakers JHJ 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

Fischer E, Keijzer W, Thielman HW, Popando O, Bohnert E, Edler EG, Jung EG and Bootsma D (1985) A ninth complementation group in xeroderma pigmentosum, XP–I. *Mutation Research* **145** 217–225

Friedberg EC (1985) *DNA Repair*. Freeman, San Francisco

Friedberg EC, Backendorf C, Burke J, Collins A, Grossman L, Hoeijmakers JHJ, Lehmann AR, Seeberg E, van der Schans GP and van Zeeland AA (1987) Molecular aspects of DNA repair. *Mutation Research* **184** 67–86

Graham FL and van der Eb A (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52** 456–467

Hoeijmakers JHJ, van Duin M, Westerveld A, Yasui A and Bootsma D (1986) Identification of DNA repair genes in the human genome. *Cold Spring Harbor Symposium on Quantitative Biology* **51** 91–101

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

Jonge de AJR, Vermeulen W, Klein B and Hoeijmakers JHJ (1983) Microinjection of human cell extracts corrects xeroderma pigmentosum defect. *EMBO Journal* **2** 637–641

Kraemer KH (1983) Heritable diseases with increased sensitivity to cellular injury. In: TB Fitzpatrick, AZ Eisen, K Wolff, IM Freedberg and KF Austen (eds), *Update: Dermatology in General Medicine*, pp 113–142. McGraw-Hill, New York

Lehmann AR (1985) Use of recombinant DNA techniques in cloning DNA repair genes and in the study of mutagenesis in mammalian cells. *Mutation Research* **150** 61–67

Oh EY and Grossman L (1987) Helicase properties of the *Escherichia coli* Uvr A B protein complex. *Proceedings of the National Academy of Sciences of the USA* **84** 3638–3642

Sancar A (1987) DNA repair *in vitro*. *Photobiochemistry and Photobiophysics* **Suppl** 301–315

Sancar A and Rupp WD (1983) A novel repair enzyme: Uvr ABC excision nuclease of *Escherichia coli* cuts a DNA strand at both sides of the damaged region. *Cell* **33** 249–260

Siciliano MJ, Carrano AV and Thompson LH (1986) Assignment of a human DNA repair gene associated with sister-chromatid exchange to chromosome 19. *Mutation Research* **174** 303–308

Siciliano MJ, Bachinski L, Carrano AV and Thompson LH (1987) Chromosomal assignments of human DNA repair genes that complement Chinese hamster ovary (CHO) cell mutants. *Human Gene Mapping* (in press)

Stefanini M, Keijzer W, Westerveld A and Bootsma, D (1985) Interspecies complementation analysis of xeroderma pigmentosum and UV-sensitive Chinese hamster cells. *Experimental Cell Research* **161** 373–380

Teo I, Sedgwick B, Kilpatrick MW, McCarthy TV and Lindahl T (1986) The intracellular signal for induction of resistance to alkylation agents in *E coli*. *Cell* **45** 315–324

Thompson LH and Carrano AV (1983) Analysis of mammalian cell mutagenesis and DNA repair using *in vitro* selected CHO cell mutants. In: EC Friedberg and BR Bridges (eds), *Cellular Responses to DNA Damage*, *UCLA Symposium on Molecular and Cellular Biology New Series*, Vol 11, pp 125–143. Alan R Liss, New York

Thompson LH, Busch DB, Brookman KW, Mooney CL and Glaser PA (1981) Genetic diversity of UV-sensitive DNA repair mutants of Chinese hamster ovary cells. *Proceedings of the National Academy of Sciences of the USA* **78** 3734–3737

Thompson LH, Mooney CL and Brookman KW (1985) Genetic complementation between UV-sensitive CHO mutants and xeroderma pigmentosum fibroblasts. *Mutation Research* **150** 423–429

Thompson LH, Salazar EP, Brookman KW, Collins CC, Stewart SA, Busch DB and Weber CA (1987) Recent progress with the DNA repair mutants of Chinese hamster ovary cells. *Journal of Cell Science Supplement* **6** 97–110

Vermeulen W, Osseweijer P, de Jonge AJR and Hoeijmakers JHJ (1986) Transient correction of excision repair defects in fibroblasts of 9 xeroderma pigmentosum complementation groups by microinjection of crude human cell extracts. *Mutation Research* **165** 199–206

Weber CA, Salazar EP, Stewart SA and Thompson LH (1988) Molecular cloning and biological characterization of a human gene, *ERCC–2*, which corrects the nucleotide excision repair defect in CHO UV5 cells. *Molecular and Cellular Biology* **8** 1137–1146

Weerd-Kastelein de EA, Keijzer W and Bootsma D (1972) Genetic heterogeneity of xeroderma pigmentosum demonstrated by somatic cell hybridization. *Nature New Biology* **238** 80–83

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

Willis AE and Lindahl T (1987) DNA ligase I deficiency in Bloom's syndrome. *Nature* **325** 355–357

Yamaizumi M, Sugano T, Asahina H, Okada Y and Uchida T (1986) Microinjection of partially purified factor restores DNA damage specifically in group A of xeroderma pigmentosum cells. *Proceedings of the National Academy of Sciences of the USA* **83** 1476–1479

(The authors are responsible for the accuracy of the references.)