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Feature Articles

The Genetic Defect in DNA Repair Deficiency Syndromes

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INTRODUCTION

DNA FUNCTIONS in living cells as a remarkably stable carrier of genetic information despite the fact that it is continuously subject to undesired, chemical alterations. Different types of radiation (eg. UV light, X-rays) and numerous chemical agents induce a wide range of lesions in DNA which interfere with its functioning. Besides the immediate, hampering effect on vital processes, transcription and replication, DNA damage may also give rise to mutations and chromosomal aberrations, leading to genetic defects, carcinogenesis, and cell death. Furthermore, it is plausible that time-dependent accumulation of DNA damage and mutations contribute to (cellular) ageing and neurodegeneration. To minimise such deleterious consequences evolution has equipped all living organisms with a complex network of repair pathways. These systems act together as a kind of intranuclear "immune system" that is able to recognise and eliminate virtually any type of lesion.

One of the major repair pathways is known as the nucleotide excision repair (NER) process. This complex system eliminates a diverse array of structurally unrelated lesions, including UV-induced cyclobutane pyrimidine dimers (CPD) and (6–4) photoproducts, a wide variety of chemical adducts and certain types of DNA crosslinks. In general terms, it consists of five steps: damage recognition, incision of the damaged strand on both sides at some distance from the lesion, excision of the injury-containing oligonucleotide, DNA resynthesis using the undamaged strand as template, and ligation.

Mechanistically, this system is best understood in the case of the bacterium *Escherichia coli* where the entire reaction is conducted by the concerted action of six proteins (Fig. 1). Briefly, a complex of two proteins UvrA and UvrB scans the helix for distorting lesions. Upon encountering such an injury the translocation stops and the UvrB subunit is attached to the DNA, inducing a specific DNA conformation. UvrC interacts with the bound UvrB molecule and the BC-complex catalyses a dual incision in the damaged strand. The helicase activity of UvrD takes care of the release of the short stretch of DNA containing the lesion, after which DNA polymerase I fills the gap and DNA ligase seals the remaining nick.

The state of knowledge about the eucaryotic pathway is still rudimentary in comparison with *E.coli*, mainly because it entails extra levels of complexity and many more components. Only some of these have been identified.

The biological relevance of DNA repair mechanisms is illustrated by a number of genetic diseases that, at the molecular level, are characterised by defective DNA repair. Xeroderma pigmentosum (XP), in which the nucleotide excision repair system is disturbed, is one of the best studied DNA repair syndromes, see [1] for a comprehensive overview on XP. Clinically, XP is a heterogeneous disorder. All patients exhibit intolerance of the skin to sun exposure resulting in progressive degenerative changes, often (but not in all cases) leading to neoplasia. Some patients have (progressive) neurological abnormalities in addition to the cutaneous abnormalities. The complexity of DNA repair mechanisms in mammalian cells is reflected in extensive genetic heterogeneity in XP. Complementation studies have shown that different genes are involved in the primary defect in different patients and even overlap is found between XP and two other nucleotide excision repair disorders: Cockayne's syndrome (CS) and trichothiodystrophy (TTD), two diseases in which cancer predisposition is not apparent. See [2] for a review on CS and TTD.

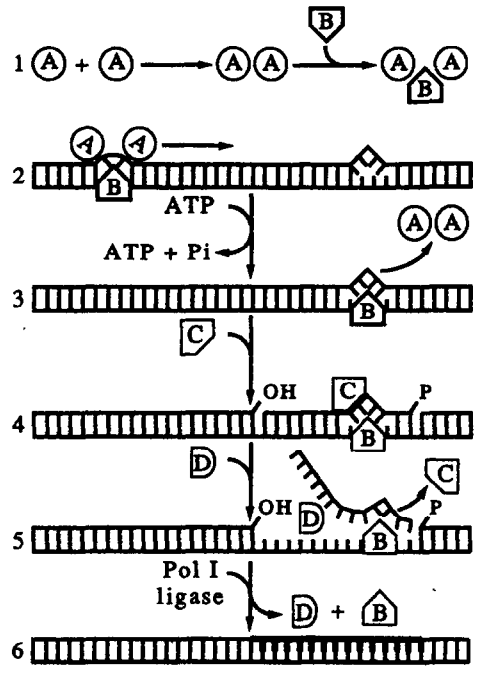

Fig. 1. A model for the nucleotide excision repair mechanism in *Escherichia coli*. In a concerted action of proteins, coded by the UvrA, B, C and D genes of *E.coli*, the DNA lesion is removed, and replaced by new DNA under the guidance of DNA polymerase I and ligase.

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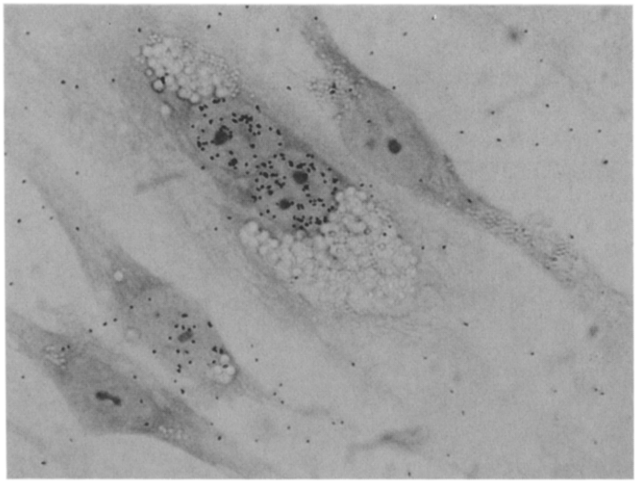

Fig. 2. Micrograph of XP complementation. Complementation after fusion of XP-C fibroblasts with XP-G fibroblasts. The XP-C cells were labelled with large and the XP-G cells with small latex beads. The XP-C/XP-G heterokaryon is identified by both types of beads. The cells were exposed to 15 J/m² UV, 2 days after fusion, and subsequently grown in the presence of tritiated-thymidine (370 kBq/ml) for 2 h followed by fixation and autoradiography. The heterokaryon shows a normal level of unscheduled DNA synthesis (UDS). The two XP-G cells do not show any labeling and the single XP-C fibroblast is only weakly labelled, reflecting the distinct degree of repair deficiency of these cells.

Recently, the first human DNA repair genes have been cloned including at least four genes involved in XP and one in CS. The cloning of these genes provides the first steps in understanding the relationship between the molecular defect and the clinical manifestations of the disorder.

COMPLEMENTATION ANALYSIS OF NER DEFICIENCY SYNDROMES AND CELL LINES

The clinical heterogeneity in XP and the marked differences in cellular expression of the excision repair defect in terms of unscheduled DNA synthesis (UDS, see Fig. 2) in different patients [3], prompted us to devise a cell fusion assay to investigate genetic heterogeneity in XP [4]. Heterokaryons formed between fibroblasts of different XP patients exposed to ultraviolet light either showed normal or nearly normal levels of UDS (patients complement each others defect and, therefore, belong to different complementation groups, Fig. 2) or they exhibit the impaired level of UDS seen in the parental XP cells (patients are in the same complementation group). Each complementation group may represent a gene that, if mutated and in a homozygous condition, causes XP.

A total of seven complementation groups has been identified in excision repair-deficient XP. This number may be a low estimate of the number of genotypes that are expected to cause deficient DNA repair in man. In comparison at least 10 distinct genes involved in nucleotide excision repair (the *RAD3* epistasis group) have been identified in *Saccharomyces cerevisiae*, and even this number is likely to be an underestimate. Some of the properties of the XP complementation groups are summarised in Table 1.

Most of the cell strains assigned to XP group A show negligible levels of UDS after exposure to UV and are extremely sensitive to UV. XP-A patients show different degrees of neurological abnormalities.

For many years XP group B consisted only of 1 patient (XP11BE). She suffered from xeroderma pigmentosum with Cockayne's syndrome. Very recently, in collaboration with H.J. Müller (Basel) we found a second family with two brothers suffering from a combination of XP and CS, whose cells could be assigned to complementation group B (Vermeulen *et al.* manuscript in preparation).

In XP-C patients the repair deficiency affects the capacity to repair the genome overall ('global genome repair'). The preferential repair of transcriptionally active DNA found to occur in rodents and humans [5] seems to be normal [6]. This may explain the residual levels of UDS in XP-C cells.

XP-D is becoming a very interesting group because of the existence of extensive clinical heterogeneity within this group. Many XP-D patients have neurological abnormalities and the onset may be delayed until the second decade. The group also encompasses patients suffering from the rare, genetic disease trichothiodystrophy (TTD). About 50% of patients suffering from TTD show, in addition to brittle hair with reduced

Table 1. XP and CS complementation groups

	Clinical properties			Repair characteristics		
Group	Skin cancer	Neurol. abnorm.	Relative frequency	UV sensitivity	Residual UDS	Remarks
XP-A	+	++	high	+++	< 5%	Different from rodent group 1–7, 11
XP-B	+/−	+++/+	very rare	++	< 10%	Combined XP/CS, identical to rodent group 3.
XP-C	+	−	high	+	15–30%	'Global genome' repair deficient, preferential repair normal.
XP-D	+	++/−	intermediate	++	15–50%	Includes patients with TTD and patients with CS, identical to rodent group 2.
XP-E	+/−	−	rare	±	> 50%	
XP-F	+/−	−	rare/interm.	+	15–30%	Repair slow but prolonged
XP-G	+/−	+++/+	rare	++	< 10%	Includes patients with CS
XP-V	+	−	high	+	wt	Defective postreplication repair, excision repair normal.
CS-A	−	++	rare	+	wt	Preferential repair defect, 'global genome' repair normal.
CS-B	−	++	high	+	wt	Preferential repair defect, 'global genome' repair normal, identical to rodent group 6

wt, wild type.

sulphur content, ichthyosis, peculiar face, mental and physical retardation (typical for TTD) also photosensitivity and reduced levels of UDS after UV exposure (typical for XP). Some of the TTD patients exhibit only a partial excision repair defect, mainly affecting the rate of removal of UV-induced 6–4 photoproducts, but not that of cyclobutane dimers [7, 8]. Complementation analysis of some of the patients belonging to this category of TTD placed them in XP group D [9]. So far this group of XP-D/TTD patients does not have skin cancer, however, they may get tumours at a later stage (the maximum age thus far was 20 years). The assignment of a patient with combined symptoms of XP and CS to XP group D [10] increased the clinical heterogeneity in the D group. The presence of three genetic disorders in one complementation group, which otherwise are present as independent entities in other patients, raises an intriguing problem. Its solution may await the cloning and characterisation of the gene or genes mutated in these diseases.

Patients assigned to complementation group E have light to moderately severe symptoms of the disease which correlate with a relatively high residual UDS level upon UV exposure of cultured fibroblasts. One of these patients (XP2RO) died from an endogenous metastatic tumour of endothelial origin. It is claimed that XP-E cells are deficient in a DNA damage-specific binding protein [11].

Complementation group F mainly consists of Japanese kindreds with mild clinical symptoms. Only in 1 case were neurological abnormalities reported and the incidence of skin cancer is low in this group.

Finally, a few patients have been assigned to complementation group G. In addition to the groups A, B and D, XP-G is the fourth group in which neurological manifestation of the disease is apparent. Some of these XP-G patients resemble patients in group B and D and have the combined symptoms of XP and CS [12].

In the classical form of Cockayne syndrome two complementation groups have been identified: CS-A and B [13] (Table 1). UDS after UV exposure of CS-A and CS-B cells is not different from normal human fibroblasts, but recovery of RNA and DNA synthesis after UV-exposure is low or absent as in XP. Recently, Venema *et al.* [14] discovered a defect in the repair of transcriptionally active genes in CS cells. "Global genome repair" was found to be normal in CS-A and CS-B, which explains the high levels of UDS. Apparently, these defects are opposite of the deficiency in XP-C.

The clinical and genetic heterogeneity in XP raises the question whether the XP complementation groups represent single gene defects, inherited in a simple Mendelian fashion, or whether more than one genetic locus is involved in each patient [15]. An obvious approach to answer this question would be the cloning of the responsible genes and their characterisation in *in vitro* [16, 17] and *in vivo* repair systems.

An important first step on the way to cloning repair genes has been the isolation of repair-deficient rodent mutant cell lines. Many of these lines are of Chinese hamster origin (CHO and V79). Complementation analysis of these mutants so far has revealed at least 11 distinct complementation groups [18, 19]. Some of their properties are summarised in Table 2. The first five groups consist of cell lines which are extremely sensitive to UV and UV-mimicking agents. Like XP, the repair defect is located in one of the early steps of the excision repair pathway. Repair in the few representatives of groups 6, 7, 9 and 10 is only partially disturbed. Cell fusion studies performed by Thompson [18] showed that human genes could complement the rodent repair defects in these mutant cell lines. These genes were designated *ERCC* (Excision Repair Cross Complementing) followed by the number of the rodent complementation group corrected by the human gene.

HUMAN NER GENES

Up till now most mammalian NER genes have been isolated by transfection of genomic DNA to UV-sensitive repair mutants followed by selection of UV-resistant transformants and retrieval of the correcting sequence. Many of the CHO cells are far superior for genomic DNA transfections compared to human cells in terms of quantity of exogenous DNA integrated into the genome and integrity of the inserted molecules. Therefore, most NER genes have been cloned using the rodent class of mutants. Table 3 lists the human NER genes cloned thus far and summarises their main properties, see [20, 21] for recent reviews. A number of features deserve specific attention.

Overlap between human repair syndromes and rodent and yeast UV-sensitive mutants

The isolation of *ERCC* genes correcting rodent repair deficiencies (*ERCC1, 2, 3, 5* and *6*) permitted to address

Table 2. Rodent NER complementation groups

Group	Representative mutant	Parental strain	Sensitivity* UV	Sensitivity* MMC	Incision deficiency	Correcting gene cloned	XP/CS equivalent
1	UV20, 43-3B	CHO	++	+++	+	ERCC1	Not existing
2	UV5, VH-1	CHO/V79	++	+	+	ERCC2	XP-D
3	UV24, 27-1	CHO	++	+	+	ERCC3	XP-B
4	UV41	CHO	++	+++	+	-	?
5	UV135, Q31	CHO, mouse lymphoma	+(+)	±	+	ERCC5	?
6	UV61, US46	CHO, mouse lymphoma	+	+	Partial	ERCC6	CS-B
7	VB11	V79	+	±	Partial	-	?
8	US31	Mouse lymph.	+	+	?	-	?
9	CHO4PV	CHO	+	+	Partial	-	?
10	CHO7PV	CHO	+	+	Partial	-	?
11	UVS1	CHO	+/++	+	+	-	?

* +: 2-5x; ++: 5–10x; +++: > 10x wild type sensitivity.

the question, whether some of them are by coincidence also implicated in XP, CS or TTD. Direct transfer of the *ERCC1* gene in representative cell lines of all XP, CS or TTD complementation groups did not result in alleviation of the NER defect in any of the mutants tested. Hence *ERCC1* is a repair gene not revealed by the known NER disorders. *ERCC2*, on the other hand, appeared to compensate for the UV-sensitivity of XP-D cells [22], whereas *ERCC3* corrected specifically the UDS defect of XP-B fibroblasts [23]. Transfections of the cloned *ERCC5* gene [24] to members of the remaining XP groups have not been published. Based on phenotype comparison XP-G is a serious candidate. Recently, *ERCC6* was found to be responsible for CS-B, the most common form of CS [25]. Mutation analysis revealed deleterious mutations in both alleles, indicating that the gene is not essential for viability. This finding indicates ERCC6 as the first eucaryotic NER protein specifically involved in the preferential repair of active genes [25]. The extensive overlap between human syndromes and rodent mutants stresses the value of the latter class for understanding the molecular basis of repair defects in man.

Except for *ERCC6* and possibly also the XP-C correcting (*XPCC*) gene (see below) all human genes have identified counterparts in the yeast system: the XP-A correcting gene (*XPAC*) is equivalent to *RAD14* [26], *ERCC1* to *RAD10* [27], *ERCC2* to *RAD3* [28], *ERCC3* to *RAD25/SSL2* [29, 30], and *ERCC5* to *RAD2* [MacInnes and Mudgett, personal communication]. Furthermore, the gene product of *XPCC* shares limited homology with that of *RAD4* [31], however, it is unclear whether these genes represent each others equivalents. The *ERCC1* gene product exhibits a remarkable pattern of homology (Fig. 3). The first 214 amino acids align with the entire (210 amino acids) *RAD10* polypeptide [27]. The remaining 83 residues of *ERCC1* display for the first part some homology with a segment of UvrA while the C-terminal 60 residues strongly resemble the C-terminus of UvrC. Apart from this domainal similarity of *ERCC1* none of the typical eucaryotic excision repair proteins demonstrates significant resemblance to UvrA-D, the key enzymes of *E.coli* NER. This remarkable observation may imply that the procaryotic system differs substantially from its eucaryotic pendant [32]. At the same time these findings underscore the significance of the yeast system as a very relevant paradigm for mammalian nucleotide excision.

Table 3. Cloned human NER genes

Human gene	Disease gene	Homology to	Functional* domain
ERCC1	?	*RAD10*; UvrA and C	Nuclear location signal, weak DNA binding
ERCC2	*XPBC*	*RAD3*	DNA binding, nucleotide binding 5' → 3' helicase (?)
ERCC3	*XPBC*	*RAD25/SSL2*	DNA-, nucleotide-, Chromatin-binding, (?) helicase, nuclear location signal
ERCC5	*XPGC*(?)	*RAD2*	Unknown
ERCC6	*CSBC*	Unknown	Helicase? Chromatin binding?
XPAC	*XPAC*	*RAD14*	Damage-specific DNA binding
XPCC	*XPCC*	Unknown	DNA binding?

* ? denotes function postulated on the basis of aminoacid sequence homology to functional domains.

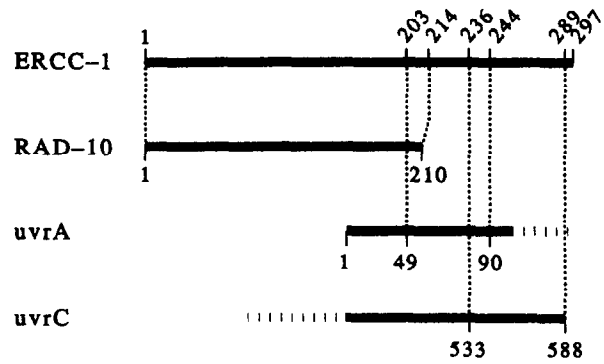

Fig. 3. Homology between *ERCC1*, *RAD10* and parts of UvrA and UvrC. The numbers indicate positions of aminoacids in the protein.

Characteristics of cloned XP and CS genes

XPAC. Tanaka and coworkers succeeded in the isolation of three repair-proficient transfectants after large-scale transfection of mouse genomic DNA into XP-A cells and subsequently cloned the correcting mouse and human genes [33, 34]. The gene encodes a protein of 273 amino acids with a predicted molecular weight of 31 kD. The protein is able to bind to ssDNA as well as dsDNA [35] and may have a preference for UV-irradiated DNA [36]. The binding to DNA is consistent with the presence of a putative Zn-finger motif that is also found in the *RAD14* gene, the yeast homolog of *XPAC*, underlining the significance of this part of the protein [26]. The damage-specific binding properties open the possibility that this protein plays a role in damage recognition.

XPCC. Recently, Legerski and Peterson [31] cloned the *XPCC* cDNA by complementing XP-C cells using an extrachromosomally replicating Epstein–Barr-derived vector that circumvents stable integration of DNA in the recipient genome. The correcting gene encodes a 3.8 kb mRNA and a highly hydrophilic protein of 823 amino acids. The presence of both highly acidic and basic domains may permit interaction with chromosomal proteins and DNA. No expression of the *XPCC* mRNA was detected in many XP-C cell lines, suggesting that this gene does not encode a vital function.

XPBC/ERCC3 and XPDC/ERCC2. The primary amino acid sequence of *ERCC2* and *3* suggests that they are helicases [28, 23]. Helicases are known to operate in a wide variety of cellular processes including replication, recombination, transcription, splicing and translation. Two helicases have already been demonstrated in the *E.coli* NER reaction: the $UvrA_2B$ complex involved in scanning for lesions and possibly in attachment of UvrB at the site of the damage, and UvrD for releasing the damage-containing oligonucleotide after incision (Fig. 1). *ERCC2* and *3* could play similar roles in mammalian NER or even function in steps not existing or disclosed in *E.coli*. It is of interest to note that intriguing parallels exist between *ERCC2* and *3* at the level of mutants, genes and proteins ([22], see also Fig. 4):

(a) *ERCC2* and *3* mutants resemble each other closely: the rodent mutants of groups 2 and 3 have a similar degree of UV sensitivity and spectrum of cross sensitivities to other DNA damaging agents, the same level of induced mutagenesis and share a preincision defect. The corresponding human XP-B and XP-D complementation

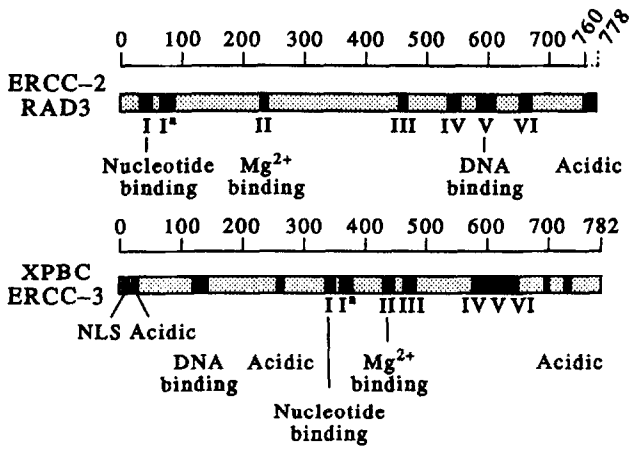

Fig. 4. The domainal structure of the *ERCC2* and *ERCC3* proteins. The predicted functional domains are indicated by black boxes; I-VI represent the seven consensus sequences of the putative helicase function of the proteins. NLS is the postulated nuclear localisation signal. The scales present the number of aminoacids.

groups display at least in some of their patients the exceptional conjunction of XP and CS symptoms.

(b) Both genes have a very similar degree of sequence conservation: they share overall ~ 50% amino acid sequence identity with their yeast cognates: *RAD3* and *RAD25*/SSL2. Particularly, the seven presumed helicase motifs reside in very conserved parts of the proteins and stand out in sequence conservation [28–30].

(c) The encoded proteins have a similar molecular weight, and are known or expected to have a DNA unwinding function because of the presence of seven 'helicase' motifs. Furthermore, both genes have a vital function in yeast [29, 30, 37] and for various reasons are suspected to have such a function in the mammalian system too.

Very recently, in collaboration with J.-M. Egly (Strasbourg) we have obtained evidence for a helicase function of the *ERCC3* protein [38]. The *ERCC3* gene product was, unexpectedly, identified as one of the components of the human BTF2/TFIIH transcription factor which is required for initiation of transcription of genes (Fig. 5a). Highly purified fractions of BTF2 possess a helicase activity strongly associated with the *ERCC3* subunit of the complex [38]. The involvement of *ERCC3* in transcription initiation probably defines the hitherto unresolved essential role of the gene. These findings also pertain to two surprising observations relating *ERCC3* to undefined steps of gene expression in yeast [30] and Drosophila [39]. Furthermore, the role of *ERCC3* in transcription may explain some of the clinical symptoms of XP-B that are not easily accounted for by the NER defect such as the severe growth defect (dwarfism and microcephaly) displayed by the XP-B patient XP11BE.

These findings uncover a functional overlap or common usage of a complex of proteins in the processes of transcription and of NER. It is tempting to speculate that this complex fulfils a similar function in both processes, e.g. it might constitute a helicase complex required to position RNA polymerase II (in transcription) and specific NER proteins (in DNA repair) on the template and to translocate them along the DNA by locally melting the double helix (Fig. 5). In *E.coli* a helicase complex is utilised for scanning of the DNA for structural distortions. An alternative possibility is that the complex induces a specific locally unwound DNA conformation. For transcription this may be necessary to permit RNA polymerase II to enter the transcribed strand. For NER such a conformation may be imperative at the site of the lesion in order to permit the incision complex to bind and act.

CSBC/ERCC6. The predicted sequence of the 1493 amino acid *ERCC6* protein again reveals the set of domains conserved between DNA and RNA helicases [25]. The protein belongs to the closely related *RAD16* subfamily that contains members of all yeast repair epistasis groups as well as proteins involved in transcription regulation. The anticipated nucleic acid unwinding activity of *ERCC6* should fit into the mechanism of the strand-selective repair of active genes that is defective in CS-B. In this perspective at least two possibilities (or a combination of the two) can be envisaged (see Fig. 6). (1) The protein may be involved in scanning the transcribed strand for a blocked RNA polymerase II thus guiding the NER machinery to lesions that thwart transcription. (2) The DNA or DNA/RNA unwinding activity may be involved in removing the stearic hindrance imposed by the transcription complex by either dissociating RNA polymerase II from the template (e.g. by unwinding the RNA/DNA duplex closely behind the transcription complex) or by pushing it either in the 3′ or 5′ direction away from the lesion [25]. Direct proof for these speculative models should come from biochemical experiments using the purified proteins and a bonafide *in vitro* repair assay system.

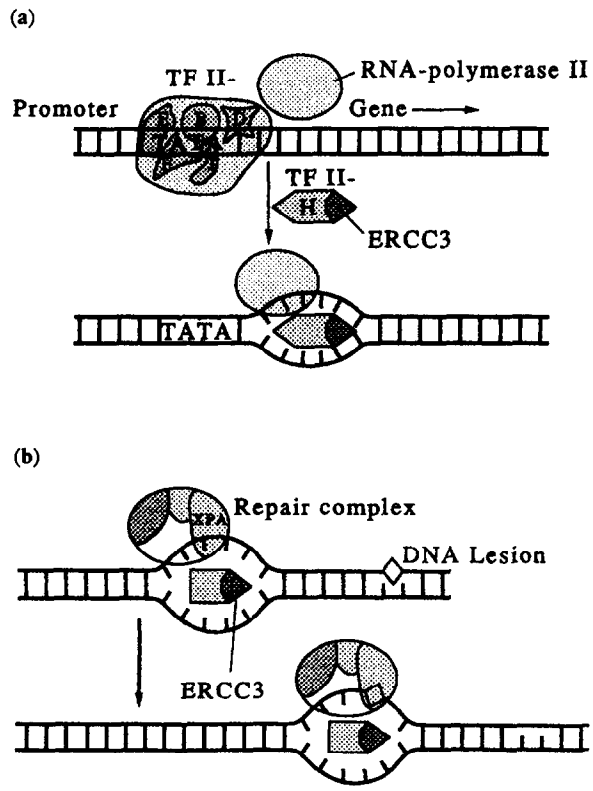

Fig. 5. Possible role of *ERCC3* in transcription (a) and DNA repair (b). As part of the TFIIH transcription factor *ERCC3* may constitute a helicase complex required to position RNA polymerase II on the template and to translocate it along the DNA by locally melting the double helix (a). In DNA repair *ERCC3* may play a similar role in positioning the specific NER proteins (XP-A protein and others) at the site of the DNA lesion.

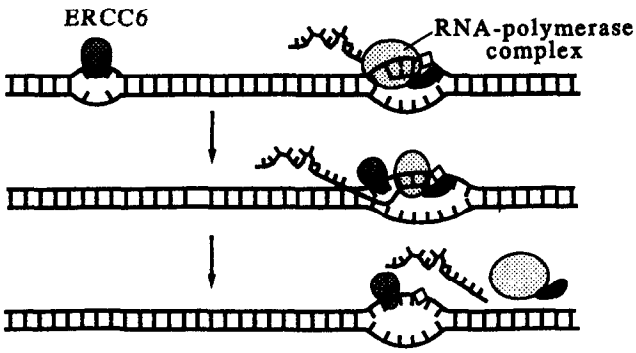

Fig. 6. Tentative model for function of *ERCC6*. *ERCC6* may be involved in scanning the transcribed strand for a blocked RNA polymerase thus guiding the NER machinery to lesions that thwart transcription. Alternatively or in addition, it may play a role in removing the stalled transcription complex, to give the repair proteins access to the damage.

THE MOLECULAR BASIS OF CLINICAL HETEROGENEITY IN XP

Four XP-genes, XPAC, XPBC, XPCC and XPDC have now been cloned and the characterisation of the genetic defects in these complementation groups is in an advanced state. Introduction of these human genes into the corresponding mutant cells results in an (almost) complete restoration of the repair defect. These observations confirm the hypothesis that XP complementation groups represent single gene defects in DNA repair, inherited in a simple Mendelian fashion. Moreover, in the case of XP-B it seems likely that a single gene defect is responsible for the combined XP/CS pattern of the disease. This does not rule out the possibility that the presence of mutations in other genes in an XP patient may contribute to the clinical manifestations of the disease. For example, as a result of the primary repair defect mutations may have taken place early in embryonic development in genes involved in the initiation or progression of tumour growth, or in genes playing a role in neurological development. One may even consider the possibility of an influence of heterozygosity for common oncogenic alleles of tumour initiating or tumour promoting genes. Similarly, as suggested by Lehmann and Norris [8], mutations in genes playing a role in immunosurveillance may influence the clinical manifestation of the disease.

The yeast *ERCC2* and *ERCC3* cognates encode essential functions. If this is true for the human genes as well, than only mutations that maintain the vital gene function would be tolerated. This is probably the situation in the XP-B patient XP11BE, where the mutation is located in the 3′ region of the gene. The extremely low frequency of XP patients in some of the complementation groups may be a consequence of the essential function of these genes.

The repair genes cloned thus far all show the presence of functional domains. It seems likely that the phenotypic expression of a mutation in such a multifunctional gene is highly dependent on the mutated site. As a consequence clinical heterogeneity is expected to occur within an XP complementation group. The analysis of mutations in different patients within a complementation group will soon throw more light on this very interesting aspect of clinical heterogeneity. The overlap of XP and CS in different XP complementation groups (B, D and G) may suggest that the genes involved in these groups have a functional domain, that plays a role in a common step in two repair pathways; one affected in CS and the other in XP. A domain encoding a common step in repair of actively transcribed DNA sequences (deficient in CS) and in "global repair" (deficient in XP-B, D and G), e.g. a helicase activity, could be a possible candidate.

PROSPECTS

Although the cloning of mammalian DNA repair genes has proved to be a difficult task, it is expected that more genes will soon be isolated. Even more difficult will be the determination of the function of the gene products. For this purpose specific *in vitro* test systems have to be developed which mimic repair activities acting in mammalian cells. The first systems of this kind have recently been established by Wood *et al.* [16] and the Sancar group [17]. To trace the relationship between molecular defect and clinical manifestation animal models for DNA repair disorders will be generated. By homologous recombination and embryonal stem cell manipulation animals with specific mutations in repair genes, whose human counterparts have been cloned, can probably be bred. These animals would permit studies towards understanding of the molecular basis of tumour susceptibility, neurological manifestations and other characteristic features of DNA repair syndromes. They could probably also be used as sensitive tools in testing the mutagenic and carcinogenic potential of agents in our environment.

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Quantum Leaps in Treatment of High-risk Breast Cancer? Prove it!

Vicky E. Jones and Derek Raghavan

THE TREATMENT of breast cancer has been the focus of intense study over the past few decades, and adjuvant trials have been conducted for more than 20 years. The benefit of adjuvant chemotherapy is established in node-positive breast cancer, with an overall reduction of 28% in the annual hazard rate for a relapse and a 16% reduction in the annual hazard rate for mortality [1]. The absolute benefit is defined by the actual risk to the patient. There is, however, controversy regarding the relative merits of disease-free (DFS) and overall survival (OS) as the best index of outcome. Similarly, the apparent impact of treatment decreases if one cites the *actual* vs. percentage reduction in relapse rate or death [2]. Endeavours to create a reliable method to predict an individual patient's risk of relapse continue, complicated by a burgeoning list of prognostic factors. Valid questions remain as to whether subgroups can be defined that have either such a good prognosis that conventional adjuvant therapy is not warranted, or are at sufficiently high risk for recurrence that other adjuvant strategies should be entertained.