All living organisms have an intricate network of systems whose role is to repair damage to DNA. One of the most important of these, nucleotide excision repair (NER), is a five-step pathway that eliminates a variety of structurally unrelated lesions, including UV-induced cyclobutane pyrimidine dimers (CPD) and (6-4) photoproducts, many types of chemical adducts and DNA crosslinks. As summarized in part I of this review, the mechanism of NER is best understood in the bacterium *E. coli*. In part II, I focus on the more complex pathway in eukaryotes, about which much less is known. I shall discuss the main features and biological impact of NER in mammals, and attempt to put these into an evolutionary and mechanistic perspective by comparing mammalian NER with the yeast system. For a comprehensive review on DNA repair in general, see Ref. 2.

**General characteristics of mammalian NER**

Each mammalian nucleus contains approximately two metres of DNA and repair systems face a formidable task in surveying this for the presence of lesions. This logistic problem seems to be reduced to some extent by the preferential (i.e. more rapid and complete) repair of transcribed sequences, a process that is carried out by a special NER subpathway also found in *E. coli* and yeast. Apparently, high priority is given to rapid resumption of transcription when this vital process is blocked by lesions in the template. The initial recognition and signalling of DNA injury in this subpathway is probably performed by RNA polymerase, whereas in the more slow and incomplete 'overall genome' NER subpathway, lesions are thought to be detected by repair complexes scanning the DNA. The screening for conformational abnormalities by RNA polymerase II may be more rigorous and this may explain why some lesions, such as CPDs, are more efficiently removed from transcribed strands than from the rest of the genome. Other lesions, such as 6-4 photoproducts, are already very efficiently recognized by the overall genome repair system. Their rate of removal from the bulk of DNA is almost the same as that achieved by transcription-coupled repair.

**Human NER-deficient syndromes**

The consequences of deficient excision repair are apparent from the clinical symptoms of the human repair disorders xeroderma pigmentosum (XP), Cockayne's syndrome (CS) and PIBIDS, a photosensitive form of the brittle hair disorder trichothiodystrophy. This is the second of a two-part review on NER. Part I (in the previous issue of *TIG*) concentrated on the main characteristics of the NER pathway of *E. coli* and yeast. Part II compares the mammalian and yeast systems, and attempts to integrate current knowledge on the eukaryotic pathway to suggest an outline for the reaction mechanism.

of CS, although the underlying cause, demyelination of neurons, is different. Growth is retarded in all CS patients, and in a subset of XP patients. Remarkably, CS patients do not have an elevated incidence of (skin) cancer. (For reviews on the clinical features of XP and CS, see Refs 4 and 5, respectively.)

PIBIDS is an acronym for a complex syndrome that comprises photosensitivity, ichthyosis (scaling of the
TABLE 1. Properties of XP, CS and PIBIDS complementation groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Skin cancer</th>
<th>Neurological abnormalities</th>
<th>Relative frequency of occurrence</th>
<th>UV-sensitivity</th>
<th>Residual UDS*</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>XP-A</td>
<td>+</td>
<td>++</td>
<td>high</td>
<td>++</td>
<td>&lt;5</td>
<td>Different from rodent groups 1-7, 11</td>
</tr>
<tr>
<td>XP-B</td>
<td>+/-</td>
<td>+++/+</td>
<td>very rare</td>
<td>++</td>
<td>&lt;10</td>
<td>Combined XP/CS Identical to rodent group 3</td>
</tr>
<tr>
<td>XP-C</td>
<td>+</td>
<td>-</td>
<td>high</td>
<td>+</td>
<td>15-30</td>
<td>Deficient in 'global genome' repair Normal preferential strand-selective repair</td>
</tr>
<tr>
<td>XP-D</td>
<td>+</td>
<td>+/-</td>
<td>intermediate</td>
<td>++</td>
<td>15-50</td>
<td>Includes patients with PIBIDS and patients with XP/CS</td>
</tr>
<tr>
<td>XP/CS</td>
<td>+</td>
<td>-</td>
<td>normal</td>
<td>+</td>
<td>&gt;50</td>
<td>Identical to rodent group 2</td>
</tr>
<tr>
<td>XP-E</td>
<td>+/-</td>
<td>-</td>
<td>rare</td>
<td>±</td>
<td>&gt;50</td>
<td>Repair slow but prolonged</td>
</tr>
<tr>
<td>XP-F</td>
<td>+/-</td>
<td>-</td>
<td>rare/intermediate</td>
<td>+</td>
<td>15-30</td>
<td>Includes patients with CS</td>
</tr>
<tr>
<td>XP-G</td>
<td>+/-</td>
<td>+++/+</td>
<td>rare</td>
<td>++</td>
<td>&lt;10</td>
<td>Defective in post-replication repair Normal NER</td>
</tr>
<tr>
<td>XP-V</td>
<td>+</td>
<td>-</td>
<td>high</td>
<td>+</td>
<td>100</td>
<td>Defective in preferential strand-selective repair 'Global genome' repair normal</td>
</tr>
<tr>
<td>CS-A</td>
<td>-</td>
<td>++</td>
<td>rare</td>
<td>+</td>
<td>100</td>
<td>Defective in preferential strand-selective repair 'Global genome' repair normal</td>
</tr>
<tr>
<td>CS-B</td>
<td>-</td>
<td>++</td>
<td>high</td>
<td>+</td>
<td>100</td>
<td>Identical to rodent group 6</td>
</tr>
<tr>
<td>TTD1BR</td>
<td>-</td>
<td>+</td>
<td>very rare</td>
<td>+</td>
<td>15</td>
<td>PIBIDS</td>
</tr>
</tbody>
</table>

*Unscheduled DNA synthesis as a percentage of wild-type activity.

skin), brittle hair, impaired intelligence, decreased fertility, and short stature. PIBIDS resembles CS in many aspects: its effects on growth, the nervous system, sexual development and photosensitivity, and in its lack of association with cancer. Brittle hair, caused by a reduced content of cysteine-rich matrix proteins, is the most prominent characteristic of trichothiodystrophy, a disorder that also affects patients who are not photosensitive (reviewed in Ref. 6).

NER deficiency is reflected at the cellular level as hypersensitivity to UV and to agents that mimic the effect of UV; in most XP and PIBIDS patients, UV-induced unscheduled DNA synthesis (UDS) is also slower or completely absent. The NER defect in classical CS is limited to the subpathway of preferential repair of (the transcribed strand of) active genes; the less efficient 'overall genome' repair system is still functional. Since preferential repair makes a relatively small contribution to the total repair synthesis, CS fibroblasts show near-normal levels of UDS. However, the preferential repair defect prevents the rapid recovery of RNA synthesis after UV exposure and this probably causes the increased UV sensitivity.

Complementation analysis shows that all three NER disorders are genetically heterogeneous; fusion of cells from different patients can complement each repair defect. An example of XP complementation is shown in Fig. 1. Seven excision-deficient XP complementation groups (designated XP-A to XP-G) have been catalogued to date (Table 1). An eighth group, called XP-variant, has normal NER but is thought to be impaired in the poorly defined postreplication repair pathway. Patients who have CS only fall into one of two complementation groups, CS-A or CS-B, whereas a number of very rare individuals with combined manifestations of XP and CS fall into XP groups B, D and G (Ref. 9 and our unpublished results; Table 1). At least two complementation groups can be distinguished for PIBIDS. Most patients have been assigned to XP group D (Ref. 11), further extending the clinical heterogeneity of this form of XP (Ref. 12). One recently identified PIBIDS patient constitutes an entirely new group. The finding that a second NER gene is associated with the clinical symptoms of PIBIDS underscores the pleiotropic and unexpected clinical effects associated with mutations in NER genes, such as the occurrence of sulfur-deficient brittle hair.

Repair deficiency is most severe in XP groups A, B and G, and affects both overall genome and preferential repair. In XP-C the excision defect is limited to the overall genome pathway, thus XP-C is the biochemical opposite of CS-A and CS-B. Since the efficiency of overall genome repair has a strong impact on mutagenesis, this may explain the high incidence of skin
# Properties of rodent NER complementation groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Representative mutant</th>
<th>Parental cell line</th>
<th>Sensitivity of mutant</th>
<th>Incision</th>
<th>Correcting gene cloned</th>
<th>XP/CS equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UV20 43-3B</td>
<td>CHO</td>
<td>++</td>
<td>++</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>2</td>
<td>UV5 VH-1</td>
<td>CHO pH V79</td>
<td>++</td>
<td>+</td>
<td>yes</td>
<td>XP-D</td>
</tr>
<tr>
<td>3</td>
<td>UV24 27-1</td>
<td>CHO</td>
<td>++</td>
<td>+</td>
<td>yes</td>
<td>XP-B</td>
</tr>
<tr>
<td>4</td>
<td>UV41</td>
<td>CHO</td>
<td>++</td>
<td>+++</td>
<td>yes</td>
<td>XP-G</td>
</tr>
<tr>
<td>5</td>
<td>UV135</td>
<td>CHO</td>
<td>+ (+)</td>
<td>±</td>
<td>yes</td>
<td>XP-E</td>
</tr>
<tr>
<td>6</td>
<td>UV61</td>
<td>CHO</td>
<td>+</td>
<td>+</td>
<td>partially</td>
<td>CS-B</td>
</tr>
<tr>
<td>7</td>
<td>VB11</td>
<td>V79</td>
<td>+</td>
<td>±</td>
<td>partially</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>U331</td>
<td>Mouse lymphoma</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>CHO4PV</td>
<td>CHO</td>
<td>+</td>
<td>+</td>
<td>partially</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>CHO7FPV</td>
<td>CHO</td>
<td>+</td>
<td>+</td>
<td>partially</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>UVS1</td>
<td>CHO</td>
<td>+/++</td>
<td>+</td>
<td>yes</td>
<td></td>
</tr>
</tbody>
</table>

*The number of plus signs indicates the sensitivity of the mutant relative to wild-type cells: +, 2-5 times greater; ++, 5-10 times greater; ++++, more than 10 times greater than wild-type sensitivity.

cancer associated with XP-C, and the lower risk for CS. As mentioned above, XP-D is a very heterogeneous group that includes XP, XP/CS and PIBIDS patients. NER is least affected in XP-E. Finally, repair in the mild XP group F appears to be slow, but ultimately quite complete.

In all XP complementation groups, the defects in repair of CPD lesions can be bypassed by introducing the prokaryotic CPD-specific endonucleases of bacteriophage T4 and Micrococcus luteus (Ref. 15, and references therein). Apparently, when incision of the damaged strand has been carried out by exogenous enzymes, the cellular repair machinery is capable of completing the task. This suggests – but does not necessarily prove – that all XP defects reside in NER steps preceding incision or affecting incision itself, and implies that the first stage of the reaction is quite complex.

## Rodent NER mutants

A second important category of mammalian mutants are the laboratory-induced, UV-sensitive rodent cell lines. These are mainly derived from Chinese hamster ovary (CHO) cell lines, and there are currently 11 NER complementation groups in this class of mutants. Their main features are summarized in Table 2 (reviewed in Ref. 17). Mutants that belong to the first five groups are extremely sensitive to UV and bulky adducts and in that respect resemble the XP groups A, B, D and G. Repair in the few representatives of the remaining groups appears to be only partially disturbed, as in CS-A and CS-B and in XP groups C, E and F. A unique characteristic of groups 1 and 4 is their extraordinary sensitivity to cross-linking agents. This suggests that the NER genes affected in these mutants act in additional systems that repair or respond to cross-link damage in DNA. As shown below, there is considerable overlap between the Chinese hamster mutants and the human NER syndromes.

## Human NER genes

So far, most mammalian NER genes have been isolated by transfection of genomic DNA into UV-sensitive rodent repair mutants, followed by selection of UV-resistant transformants and retrieval of the correcting sequence. Recently, however, the use of cDNA expression libraries in extrachromosomally replicating vectors based on the Epstein-Barr virus has circumvented many of the problems that previously limited the use of human cells for transfection.

The human genes correcting rodent repair defects are called ERCC genes, for excision repair cross-complementing rodent repair deficiency genes. The number refers to the rodent group that is corrected. Table 3 lists the human NER genes cloned thus far and summarizes their main properties (reviewed in Ref. 20). Some general aspects are discussed below.

### Expression

The cloned mammalian NER genes are weakly and constitutively expressed in various cells and tissues. No significant UV-inducibility has been observed for XPA¢, ERCC1 and ERCC3.

### Overlap between human repair syndromes and rodent mutants

Introduction of the ERCC2, ERCC3 and ERCC6 genes into human NER-deficient cell lines alleviated specific defects in cells from groups XP-D, XP-B and CS-B respectively. The correspondence between ERCC6 and CS-B identifies ERCC6 as the first eukaryotic NER
### Table 3. Properties of cloned human NER genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Gene size (kb)</th>
<th>No. of amino acids in protein</th>
<th>Homologous gene in S. cerevisiae</th>
<th>% identity/similarity to S. cerevisiae gene</th>
<th>Properties of protein*</th>
</tr>
</thead>
<tbody>
<tr>
<td>XPAC</td>
<td>9q34</td>
<td>-25</td>
<td>273</td>
<td>RAD14</td>
<td>27/50</td>
<td>Zn²⁺ finger, binds ss and UV-irradiated dsDNA</td>
</tr>
<tr>
<td>XPBC/ERCC3</td>
<td>2q21</td>
<td>-45</td>
<td>782</td>
<td>RAD25/SSL2</td>
<td>55/72</td>
<td>DNA binding helicase, Chromatin binding? Role in transcription</td>
</tr>
<tr>
<td>XPCC</td>
<td></td>
<td>823</td>
<td></td>
<td>RAD4?</td>
<td></td>
<td>Homology to RAD4 limited to one segment of the protein</td>
</tr>
<tr>
<td>XPDC/ERCC2</td>
<td>19q13.2</td>
<td>-20</td>
<td>760</td>
<td>RAD3</td>
<td>52/72</td>
<td>DNA binding? DNA helicase? Essential function?</td>
</tr>
<tr>
<td>XPGC/ERCC5</td>
<td>13q32-33</td>
<td>-32</td>
<td>1186</td>
<td>RAD2</td>
<td>24/39</td>
<td>Complexed with ERCC4, ERCC11, XPCC</td>
</tr>
</tbody>
</table>

*Question marks indicate characteristics inferred on the basis of the predicted amino acid sequences of the proteins.

#### Encoded functions

**XPAC.** The purified XPAC protein has an affinity for single-stranded (ss) DNA as well as UV-irradiated double-stranded (ds) DNA and contains a predicted DNA-binding zinc-finger domain. Its preference for binding to UV-induced lesions may implicate XPAC in damage recognition. From the nature of the XP-A repair defect as measured in the in vitro NER assay (see below), the protein is expected to function in a preincision step of NER. In a number of XP-A patients, the XPAC gene contains aberrant splicing signals and nonsense codons, many of which are expected to completely inactivate it. In general, there is a good correlation between the severity of the mutation and the severity of the clinical features.

**XPCC.** Unfortunately, the predicted 823 amino acid sequence of XPCC does not provide interpretable clues to its function. The absence of XPCC mRNA in many XPC patients suggests that complete inactivation of the gene is not lethal.

**ERCC1.** Deletion analysis of ERCC1 indicates that the five carboxy-terminal amino acids of the protein residing in a region that has significant homology with the carboxyl terminus of UvrC, are indispensable for its repair function (our unpublished observations). The corresponding part of UvrC is similarly essential. Another parallel between ERCC1 and UvrC is that absence of the entire amino-terminal region appears not to have a drastic effect on the repair function of the proteins. There is recent evidence that ERCC1 resides in a complex with several other NER proteins: the products of the as yet uncloned ERCC4, ERCC11.

#### Overlap between mammalian and yeast genes

Except for ERCC5 and possibly XPCC, all human genes have identified counterparts in the yeast system (Table 3), with the level of sequence identity between corresponding yeast and mammalian proteins ranging from 25% to over 50%. XPCC may be equivalent to RAD4, but the region of similarity comprises only one quarter of the protein. The absence of XPCC mRNA in many XP-C patients suggests that complete inactivation of the gene is not lethal.

**ERCC1.** Deletion analysis of ERCC1 indicates that the five carboxy-terminal amino acids of the protein residing in a region that has significant homology with the carboxyl terminus of UvrC, are indispensable for its repair function (our unpublished observations). The corresponding part of UvrC is similarly essential. Another parallel between ERCC1 and UvrC is that absence of the entire amino-terminal region appears not to have a drastic effect on the repair function of the proteins. There is recent evidence that ERCC1 resides in a complex with several other NER proteins: the products of the as yet uncloned ERCC4, ERCC11.
and XPFC genes (our unpublished results). Since group 1 and 4 mutants, unlike other rodent NER mutants, are extremely sensitive to crosslinking agents, the ERCC1 complex may also play a role in the repair of a subset of DNA cross links. One of the partners of ERCC1 in this complex could be the yeast homologue, RAD10 (Ref. 1). The role of these proteins in mitotic recombination, and the presumed ability of RAD1 to remove regions of non-homology from the 3' end of recombining DNA molecules, are compatible with the idea that this complex may be responsible for, or associated with, incision, and (when required for the elimination of interstrand cross links) with an additional recombination step.

ERCC2 and ERCC3. The primary amino acid sequences of ERCC2 and ERCC3 suggest that they are helicases23,30. Helicases are known to operate in a wide variety of cellular processes including replication, recombination, transcription, splicing and translation (reviewed in Ref. 40). Two helicases have already been demonstrated in the E. coli NER reaction: the UVrA/B complex involved in scanning for lesions and possibly in attachment of UVrB at the site of the damage, and UvrD, involved in releasing the damaged oligonucleotide after incision. ERCC2 and ERCC3 could play similar roles in mammalian NER, or could even function in steps that do not exist, or have not been discovered, in E. coli. It is interesting to note that there are intriguing parallels between ERCC2 and ERCC3 (Ref. 23):

(1) ERCC2 and ERCC3 mutants are very similar;
(2) The proteins have a similar degree of sequence conservation with their yeast cognates RAD3 and RAD25/SSL2 (Refs 30–32);
(3) The proteins are of similar size and their seven 'helicase' motifs suggest a DNA-unwinding function (Fig. 2). Very recently, evidence for helicase activity of ERCC3 has been obtained41. The ERCC3 gene product was identified by Egly and co-workers42 as one of the components of the human TFIIH/BTF2 transcription factor, which is required for initiation of transcription of genes by RNA polymerase II. Highly purified fractions of TFIIH have an ATP-dependent helicase activity strongly associated with the ERCC3 subunit of the complex43. Other evidence for a role for ERCC3 in gene expression has come from the yeast gene RAD25/SSL2 (Ref. 1) and from the work of Mounkes et al.32, who have identified the Drosophila homologue of ERCC3 as the gene mutated in haywire mutants. These mutants show various defects including male-stereility, which is thought to be caused by reduced expression of the B2t tubulin gene required for normal spermatogenesis32.

A role for ERCC3 in transcription (and subtle defects in this process caused by mild mutations in the gene) may explain some of the clinical symptoms of XP-B that are not easily accounted for by the NER defect. These include the severe growth defect and the dysmyelination of neurons. The latter phenotype may be related to the expression level of the myelin basic protein that, at least in mice, critically determines the degree of CNS myelination44. This suggests that defects in group XP-B represent, at least in part, a transcription syndrome.

An important question remains: how can ERCC3 be involved in both transcription and nucleotide excision? Two options can be envisaged. The simplest interpretation is that ERCC3 catalyses a DNA unwinding step both in transcription initiation and in NER. A second possibility is that ERCC3 functions only in transcription initiation, and that some mutations in the protein have an adverse effect on the transcription of one or more DNA repair genes, indirectly resulting in a NER defect31. One would then have to assume that the strict dependence of such a repair gene on optimal functioning of ERCC3 has been preserved in eukaryotic evolution from yeast to Drosophila and to humans.

Considering the remarkable similarities between ERCC2 and ERCC3, it is tempting to speculate that these proteins act together in the same step of transcription initiation and the NER reaction, perhaps forming a helicase complex required for local melting of the double helix, to allow entry of RNA polymerase or NER proteins, and/or their translocation along the DNA template44. Evidence that supports the latter function for RAD3 has been reported recently45. As schematically depicted in Fig. 3, the XPAC protein and/or the protein mutated in XP-E (Ref. 45) might also constitute part of such a complex. It is also possible that ERCC2 and ERCC3 are required for release of the damage-containing oligonucleotide after incision, and turnover of bound NER proteins.

ERCC3. Homology searches of the 1186 amino acid ERCC5 (XPJC) sequence revealed an overall similarity with the RAD2 protein. In addition, two regions of
E. coli, generating a 29 bp lesion-containing oligonucleotide. After incision, DNA synthesis is apparently carried out by DNA polymerase δ or ε, since this reaction is dependent on the proliferating cell nuclear antigen (PCNA). DNA polymerase δ is normally implicated in leading strand synthesis of semi-conservative DNA replication. These findings therefore reveal that part of the replication machinery is also used in repair reactions, and illustrate that there is considerable overlap between these fundamental processes that metabolize DNA.

Concluding remarks

The known or postulated properties of the eukaryotic NER genes, and analogy with the main steps of the E. coli pathway, suggest the following scenario for NER in mammals:

1. The ERCC2/ERCC3/XPAC and possibly XPEC proteins are involved in detection of lesions, with the helicases scanning the DNA helix, and XPAC and XPEC recognizing the lesion. ERCC2 may also be implicated in this step.
2. Upon encountering a lesion, the complex locally denatures the DNA; this specific denatured conformation is recognized by the ERCC1/ERCC4/ERCC11/XFPC complex, which performs the dual incision. The Uvr C-like carboxy terminus of ERCC1 could play an important role in this reaction.
3. HSSB, PCNA and DNA polymerase are involved in removal of the damage-containing oligonucleotide, turn over of the incision complex and resynthesis of DNA.

Future research will undoubtedly reveal whether this speculative scenario bears any relation to the actual situation within the cell. Although complete elucidation of the eukaryotic NER reaction mechanism may still seem far away, the contours of the systems are beginning to emerge from the pieces of the puzzle that have already been identified; the yeast model is expected to be of great importance because of its versatility and relative simplicity. Furthermore, valuable tools are available in the form of in vitro repair assay systems.

Finally, we should soon see the NER genes that have already been cloned being used to generate repair-deficient transgenic mice by targeted gene inactivation. Such mouse models may yield valuable insight into how the molecular defect is translated into clinical features, such as predisposition to cancer, and into the origin of the clinical heterogeneity observed in human disorders. They may also prove very important for studies related to ageing, neurodegeneration and assessment of the genotoxicity of mutagenic agents.

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References
1 Hoeijmakers, J.H.J. (1993) Trends Genet. 9, 175–177
2 Friedberg, E.C. (1985) DNA Repair; Freeman
16 Riboni, R. et al. (1992) Cancer Res. 52, 6090-6091
35 Eker, A.P.M. et al. (1992) Mutat. Res. 274, 211–224
36 Tanaka, K. et al. (1990) Nature 348, 75-76
41 Shaffer, L. et al. (1993) Science 260, 58-63

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