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Xeroderma pigmentosum complementation group E and UV-damaged DNA-binding protein

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Abstract

UV-damaged DNA-binding protein (UV-DDB) is composed of two subunits, DDB1 (p127) and DDB2 (p48). Mutations in the *DDB2* gene inactivate UV-DDB in individuals from complementation group E of xeroderma pigmentosum (XP-E), an autosomal recessive disease characterized by sun sensitivity, severe risk for skin cancer and defective nucleotide excision repair. UV-DDB is also deficient in many rodent tissues, exposing a shortcoming in rodent models for cancer. In vitro, UV-DDB binds to cyclobutane pyrimidine dimers (CPDs), 6–4 photoproducts and other DNA lesions, stimulating the excision of CPDs, and to a lesser extent, of 6–4 photoproducts. In vivo, UV-DDB plays an important role in the p53-dependent response of mammalian cells to DNA damage. When cells are exposed to UV, the resulting accumulation of p53 activates *DDB2* transcription, which leads to increased levels of UV-DDB. Binding of UV-DDB to CPDs targets these lesions for global genomic repair, suppressing mutations without affecting UV survival. Apparently, cells are able to survive with unrepaired CPDs because of the activity of bypass DNA polymerases. Finally, there is evidence that UV-DDB may have roles in the cell that are distinct from DNA repair.

Keywords

XP-E; UV-DDB; DDB1; DDB2; p48; p127; Global genomic repair; Nucleotide excision repair

1. Introduction

Despite its rare incidence of 1 in every 250,000 live births, xeroderma pigmentosum (XP) is an autosomal recessive disease that has been the focus of much interest because of its striking phenotype of sun sensitivity, severe risk for skin cancer and defective nucleotide excision repair (NER) [14].

NER removes damaged DNA bases by excising the oligonucleotide containing the damaged base. DNA polymerase and ligase activities then fill in the gapped DNA. NER recognizes a broad range of different DNA lesions, many of which are associated with human cancer. UV-induced cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts are associated with skin cancer; cisplatin intrastrand cross-links produced during cancer treatment are themselves carcinogenic; benzo(a)pyrene adducts induced by tobacco smoke cause lung cancer; and aflatoxin adducts lead to liver cancer.

NER consists of two subpathways, transcription-coupled repair (TCR) and global genomic repair (GGR) [17]. TCR refers to the efficient repair mechanism for DNA that is actively

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transcribed. Even within a transcribed gene, the transcribed strand of DNA is repaired more efficiently than the non-transcribed strand [70]. GGR refers to the less efficient mechanism for repairing non-transcribed DNA [50].

Seven genetic complementation groups for XP have been defined by cell fusion studies. Individuals from groups A, B, D, F and G are extremely sun sensitive and pre-disposed to skin cancer. Some individuals from these complementation groups have grave neurological abnormalities, including mental retardation and microcephaly. The genes for groups A, B, D, F and G are required for both TCR and GGR. Individuals from groups C and E lack neurological abnormalities and show less extreme sun sensitivity. The genes for groups C and E are required for GGR, but not TCR [73,78].

Cockayne's syndrome (CS) is an autosomal recessive disease characterized by sun sensitivity, mental retardation and short stature [14]. Unlike XP, CS is not associated with skin cancer. The genes for CS groups A and B (CSA and CSB) are required for TCR, but not GGR. The CSA and CSB proteins are believed to somehow couple the arrest of RNA polymerase II at DNA lesions to the NER pathway [9].

XP group E (XP-E) has the mildest degree of sun sensitivity of all XP groups, although these individuals are at high risk for skin cancer. Some XP-E individuals have not manifested the degree of abnormal pigmentation, dry skin and sun sensitivity usually required to make the clinical diagnosis of XP. Instead, they were assigned to XP-E retrospectively because they developed multiple skin cancers and were later found to lack UV-damaged DNA-binding protein (UV-DDB) [26].

The relatively mild clinical phenotype of XP-E reflects its cellular phenotype. XP-E fibroblasts are significantly less sensitive to UV than fibroblasts from other complementation groups. XP-E fibroblasts also show a relatively mild defect in unscheduled DNA synthesis, which measures DNA repair by the incorporation of radio-labeled nucleotides after cells are UV exposure.

A number of confusing issues have been associated with complementation group E. For example, cells from group E were initially found to lack a UV-DDB activity [5], but subsequent studies found that other cells assigned to group E expressed UV-DDB [34]. Cell free systems for NER either did not require UV-DDB or were inhibited by its addition [1,31].

Recently, considerable progress has been made in understanding XP-E and UV-DDB. Many of the most troublesome questions have now been answered. Here, we will review the current literature for XP-E and UV-DDB, offer a perspective for the complex interplay between UV-DDB and several other protein partners, and define areas for future investigation.

2. XP-E is caused by mutation of the DDB2 subunit of UV-DDB

In 1988, Chu and Chang used an electrophoretic mobility shift assay (EMSA) to identify a UV-damaged DNA-binding protein (XPE–BF) that was defective in cells from two consanguineous XP-E patients [5]. Interestingly, it is very likely that the same protein was isolated earlier by Feldberg and Grossman [11]. The UV-damaged DNA-binding protein has also been called UV-DDB, a nomenclature we will adopt here. UV-DDB was present in cells from normal individuals and XP patients in the other six complementation groups. Subsequent reports noted that normal levels of UV-DDB could be found in several putative XP-E patients [29,34]. This biochemical heterogeneity in XP-E has remained an unresolved problem.

Keeney et al. purified UV-DDB from HeLa cells to apparent homogeneity as a 127 kDa polypeptide [32]. Interestingly, some but not all of the most highly purified fractions also contained a 48 kDa polypeptide. The genes for both polypeptides, *DDB1* (GeneBank U18299)

and *DDB2* (GeneBank U18300), were cloned and found to encode polypeptides of 1140 and 428 amino acids, with predicted molecular weights of 127 and 48 kDa, respectively [10,71]. In the literature, p125, p127 and DDB1 have been used interchangeably, as have p48 and DDB2. To avoid confusion, we propose the universal adoption of DDB1 and DDB2 to describe the subunits of UV-DDB.

The *DDB1* locus mapped to 11q12–q13, and the *DDB2* locus mapped to 11p12–p11 [10]. When purified preparations containing *DDB1* and *DDB2* were microinjected into XP-E cells lacking UV-DDB, unscheduled DNA synthesis increased two-fold up to wild-type levels, demonstrating a role in NER [33].

XP-E individuals lacking UV-DDB have mutations in *DDB2* that lead to single amino acid substitutions or a C-terminal truncation, as shown in Table 1 [26,55,56]. By contrast, individuals with intact UV-DDB, including normal individuals and individuals originally assigned to XP-E, had no mutations in either *DDB1* or *DDB2*.

The association of mutations in *DDB2* with loss of UV-DDB suggested that *DDB2* is required for successful expression of UV-DDB. One way of proving that *DDB2* is required for UV-DDB would be to transfect XP-E cells with an expression vector for *DDB2* and show restoration of the binding activity. However, XP-E cells are not amenable to transfection. On the other hand, hamster cell lines also lack UV-DDB and transfection of the hamster cells with wild-type *DDB2* activated UV-DDB [24]. Transfection with *DDB2* containing the R273H and K244E mutations found in XP-E cells (Table 1) failed to activate UV-DDB. Therefore, these amino acid substitutions inactivate the DDB2 protein.

The standard method for assigning XP patients to complementation groups is a particularly difficult procedure for group E. XP-E cells show a mild deficiency in UV-induced unscheduled DNA synthesis, with levels of approximately 50% [25]. To determine whether a new patient should be assigned to group E, cells from the patient are fused to XP-E cells and the multinucleated cells are assessed for UV-induced unscheduled DNA synthesis. Successful complementation in the fused cells indicates that the patients belong to different complementation groups. For group E, the complementation signal is only a two-fold increase in unscheduled DNA synthesis. In the face of experimental uncertainties, this signal can be difficult to distinguish from unsuccessful complementation.

There have been 20 reported cases of XP-E in the literature. UV-DDB was absent in seven cases, present in nine and unknown in the remaining four cases [25,29,34,59]. The 16 cases that have been tested for UV-DDB are shown in Table 1. Recently, three patients originally assigned to group E were subsequently re-assigned to XP group F (XP89TO), XP variant (XP43TO) and UV-sensitive syndrome (XP24KO) [25]. In addition, three other putative XP-E patients with intact UV-DDB (XP70TO, XP80TO and XP81TO) were mistakenly assigned to group E based on failure to complement XP24KO (UV-sensitive syndrome). Another putative XP-E patient with intact UV-DDB, XP26KO, cannot be reliably assigned because cell fusions failed to complement both XP24KO (UV-sensitive syndrome) and XP2RO (XP-E). Thus, UV-DDB is absent in seven XP-E patients and present in only two remaining putative XP-E patients. However, one of the XP-E cell lines lacking UV-DDB (XP82TO) was able to induce low levels of UV-DDB after UV exposure [27]. Interestingly, XP82TO showed mild clinical manifestations and had not developed any skin cancers by age 41.

Because cell fusion experiments represent daunting technical problems for group E, we endorse the proposal that XP-E patients be defined by the presence of mutations in the *DDB2* gene [6,27]. Linking XP-E to mutations in the *DDB2* gene will eliminate the uncertain terminology that has complicated XP-E. So far, all of the reported mutations in *DDB2* have led to inactivation of UV-DDB. However, it is possible that other mutations will be discovered that

preserve UV-DDB but still interfere with its DNA repair function. Mutations in the *DDB1* gene may also lead to inactivation of UV-DDB, although patients with *DDB1* mutations have not yet been reported (Table 1). If such patients are discovered, they should be assigned to a new complementation group.

3. The DDB1 and DDB2 subunits of UV-DDB

The DNA-binding activity of UV-DDB co-purifies with a 127 kDa polypeptide, DDB1 [2, 21,32]. However, highly purified fractions of UV-DDB may contain DDB1 alone or DDB1 plus DDB2. Apparently, DDB2 alone does not have DNA-binding activity. Fractions containing predominantly DDB1 can bind to damaged DNA even when those fractions contain very low levels of DDB2 [32].

When fractions enriched for DDB1 at the expense of DDB2 are incubated with a DNA fragment synthesized to contain a single 6–4 photoproduct, binding is observed with a defined footprint [64]. By contrast, fractions containing both DDB1 and DDB2 in a stoichiometric heteroduplex produce an altered footprint of approximately the same overall size but with an altered pattern of DNAse I hypersensitive sites. Thus, both DDB1 alone and DDB1 in a complex with DDB2 will bind to UV-damaged DNA.

These biochemical observations must be reconciled with observations in intact cells that expression of wild-type DDB2 is required for active UV-DDB. Hamster cell lines expressing DDB1 in the absence of DDB2 do not contain detectable levels of UV-DDB [24]. A possible explanation is that DDB2 is required to activate a latent binding activity in DDB1, but once DDB1 acquires its binding activity, DDB2 is dispensable.

Activation of DDB1 must be post-transcriptional. Untransfected hamster cell lines express significant levels of *DDB1* mRNA and these levels remain unchanged with the activation of binding activity by DDB2 transfection [24]. In vitro transcription and translation of *DDB1* cDNA in rabbit reticulocyte extracts produces a polypeptide with only trace levels of binding activity [23]. Even co-translation of DDB1 and DDB2 in the reticulocyte extracts fails to produce significant binding activity. Co-expression of DDB1 and DDB2 in *Escherichia coli* also fails to produce binding activity [18]. On the other hand, co-expression of DDB1 and DDB2 in baculovirus generates active UV-DDB [82]. The activation of DDB1 could occur by a direct interaction with DDB2 or by an indirect interaction. For example, DDB1 might be phosphorylated by a kinase that acts on DDB1 only in the presence of DDB2. Interestingly, when cell extracts are treated with calf intestine alkaline phosphatase, the binding activity of UV-DDB is abolished (Hwang and Chu, unpublished data).

4. There is strong evolutionary conservation for *DDB1*, but not *DDB2*

The *DDB1* gene is strongly conserved in a broad range of eukaryotes (Fig. 1). The DDB1 amino acid sequences in humans (*DDB1*, GeneBank U18299) and mice (*DDB1*, GeneBank AF159853) are 99.5% identical. Homologs of *DDB1* are found in the fruit fly *Drosophila melanogaster* (GeneBank AF132145), the plant *Arabidopsis thaliana* (CAA17529), the nematode *Caenorhabditis elegans* (CAA92824), the slime mold *Dictyostelium discoideum* (RepE, S71092) and the fission yeast *Schizosaccharomyces pombe* (CAB11219). It is not yet known whether these organisms contain proteins that bind to UV-damaged DNA. DDB1 contains three regions of unknown function that are strongly conserved: amino acid regions 301–337, 345–413 and 627–695 [88]. Recently, a hidden Markov model of structurally conserved repeats detected 16 to 21 repeats in DDB1 and its homologs [54]. These structured repeats are about 40 residues in length and only weakly conserved at the sequence level. They suggest that DDB1 contains two to three β-propeller domains, which are structurally related to the β-propellers of WD40 repeat proteins.

Although a DDB1 homolog exists in *S. pombe*, none can be found in the budding yeast *Saccharomyces cerevisiae*. On the other hand, photolyase exists in *S. cerevisiae*, but not in *S. pombe*. Thus, each of the two yeast species contains either DDB1 or photolyase, but not both. At the protein level, wild-type *S. cerevisiae* extracts contain a binding activity for UV-damaged DNA that is attributable to photolyase, while extracts from photolyase deletion mutants contain no other detectable binding activity [61]. Thus, photolyase and DDB1 share similar functions in targeting CPDs for repair and yeast appear to have evolved to utilize only one of the two genes.

Homologs of human DDB2 are found only in other mammals. The DDB2 amino acid sequences in humans (GeneBank U18300) and mice (GeneBank AAK16180) are only 73% identical. Homologs of DDB2 do not exist in other eukaryotes, including several organisms that have been completely sequenced, including *Drosophila, C. elegans, S. pombe* and *S. cerevisiae* (Fig. 1). Since DDB2 is required for the expression of active UV-DDB in mammals, the lack of a DDB2 homolog suggests that UV-DDB is absent in these other organisms.

The DDB2 protein contains seven WD40 repeats [54], one of which was reported previously [24]. The WD40 repeats in DDB2 are homologous to the WD40 repeats in a family of proteins involved in the re-organization of chromatin. Included in this subfamily are subunits of chromatin assembly factor CAF-1 in yeast, *Drosophila* and human [30,76,79]. Human CAF-1 is required for re-assembly of chromatin coupled to NER [16]. In addition, DDB2 associates with the CBP/p300 family of histone acetyltransferases [8]. The β-propeller repeats in DDB1 are related to WD40 repeats and it is notable that both DDB2 and DDB1 associate with the histone acetyltransferase complex STAGA [47]. These observations raise the possibility that UV-DDB plays a role in re-modeling chromatin at sites of DNA damage to facilitate GGR. The presence of a similar WD40 motif in the CSA protein suggests that CSA might play a similar role for TCR.

It is interesting that DDB1 homologs exist in *Drosophila, C. elegans* and *S. pombe*, while DDB2 homologs do not (Fig. 1). Like DDB1, XPD is highly conserved, with homologs in *S. cerevisiae* as well as *Drosophila, C. elegans* and *S. pombe*. The XPD protein plays critical roles in both transcription and DNA repair. Perhaps DDB1 has a second function not related to DNA repair. This possibility is discussed more fully in Section 14.

5. UV-DDB binds to a broad range of DNA lesions

UV-DDB binds with high affinity to UV-damaged DNA, exhibiting a strong preference over undamaged DNA [21]. The most abundant lesions induced by UV are *cis,syn*-CPDs followed by 6–4 photoproducts. UV-DDB binding correlates with the degree of distortion produced by these lesions, having the greatest affinity to 6–4 photoproducts [2,20,64,74]. UV-DDB binds less strongly to CPDs [21], with a lower affinity for the more abundant *cis,syn*-CPD than for the *trans,syn*-CPD [64], reflecting the degree of distortion produced by these stereoisomers.

UV-DDB will also bind to a broad range of other forms of damaged DNA, including DNA damaged by cisplatin, nitrogen mustard, psoralen, abasic sites, and single-stranded DNA [3, 5,15,62]. On the other hand, no significant binding affinity was detected for 8-oxo-guanine, ^{6}O-methylguanine, T·C or T·T mismatches or nicked DNA [15]. These results suggest that UV-DDB is capable of binding to several forms of damaged DNA that have yet to be identified. Table 2 summarizes the DNA lesions recognized by UV-DDB.

6. UV-DDB expression is highly variable in different mammals and tissues

UV-DDB is relatively abundant in every human tissue examined to date. By contrast, UV-DDB is absent or present at very low levels in many rodent tissues. Failure to express UV-

DDB in hamster cell lines is due to silencing of the *DDB2* gene by methylation [24]. UV-DDB expression can be activated in hamster cell lines by treatment with the demethylating agent 5-azacytadine or by transfection with an expression vector for *DDB2*. In mouse cells, UV-DDB is probably silenced by other mechanisms but its expression is also activated by *DDB2* transfection. In fact, rodent genomes contain the *DDB1* and *DDB2* genes, but expression of UV-DDB is suppressed in many tissues. In the mouse, *DDB2* mRNA levels are relatively low in skin, brain, lung, muscle and heart, but relatively high in testes, liver and kidney [72].

Why do many rodent tissues fail to express UV-DDB? Rodents do not express UV-DDB in skin, but a similar defect in humans leads to XP-E and a high risk for skin cancer. Perhaps rodents do not have as great a need for UV-DDB because their skin is already protected by fur and they are nocturnal animals. Rodents may have evolved to express less UV-DDB because its expression may be disadvantageous in some way. UV-DDB binds to many forms of damaged DNA (Table 2) and its binding to some lesions may interfere with other repair pathways. Interference between DNA repair pathways has been observed before. For example, yeast photolyase will bind to cisplatin-damaged DNA and interfere with the NER pathway that would otherwise remove the damage [13].

7. UV-DDB enhances global genomic repair in intact cells

XP-E cells lacking UV-DDB have defective GGR but normal TCR [22]. The GGR defect is most pronounced for CPD repair, but there is also a mild defect in the repair of 6–4 photoproducts. Like XP-E cells, hamster cell lines do not express UV-DDB and do not perform any detectable GGR of CPDs [24]. Unlike XP-E cells, the hamster cell lines repair 6–4 photoproducts with a high level of efficiency. The association of deficiencies in UV-DDB and CPD repair suggested that UV-DDB might be involved in the GGR of CPDs.

To definitively establish the role of UV-DDB in GGR, a *DDB2* cDNA expression vector was stably transfected into hamster cell lines [73]. Expression of *DDB2* activated UV-DDB and conferred efficient GGR of CPDs to levels seen in wild-type human cells. The expression of *DDB2* had no effect on GGR of 6–4 photoproducts and no effect on TCR of CPDs. Thus, UV-DDB is required for the GGR of CPDs.

There is evidence that UV-DDB plays an accessory role in targeting 6–4 photoproducts for GGR in vivo. UV-DDB has a higher affinity for 6–4 photoproducts than for CPDs. The repair of 6–4 photoproducts is mildly defective in XP-E patients [22]. Indeed, a non-sense mutation causing a C-terminal truncation in *DDB2* is associated with a defect in 6–4 photoproduct repair in the face of normal CPD repair [26]. Mammalian cells may have a mechanism for recognizing 6–4 photoproducts that does not require UV-DDB. This mechanism is more efficient in hamster than in human cells, since the absence of UV-DDB has no effect on 6–4 repair in hamster cells, but a detectable effect in human cells. In fact, transfection of *DDB2* increases 6–4 photoproduct repair in human cells [82], but not in hamster cells [73].

8. UV-DDB has no effect on UV survival but suppresses mutagenesis

Although the expression of UV-DDB in hamster cell lines strongly stimulates the repair of CPDs, UV-DDB has no detectable effect on UV survival. This striking result demonstrates that the failure to remove CPDs from non-transcribed DNA is not lethal. Indeed, the bypass DNA polymerase (pol η) is capable of replicating DNA past CPDs [48]. It is likely that several bypass polymerases, in addition to pol η, permit replication past CPDs, which produce relatively mild distortion in the DNA. As a result, the replication fork is not blocked and unrepaired CPDs are not sufficient to cause cell death.

Unlike hamster cells, human XP-E cells lacking UV-DDB are sensitive to UV, although not to the same degree as other XP cells. This difference between hamster and human cells is correlated with different efficiencies in the repair of 6–4 photoproducts. The absence of UV-DDB in human XP-E cells is associated with a mild defect in the repair of 6–4 photoproducts, but the absence of UV-DDB in hamster cells has no such effect. Thus, the mechanism for recognizing 6–4 photoproducts in the absence of UV-DDB appears to be more efficient in hamster cell lines than in human cells.

Expression of UV-DDB by *DDB2* transfection of hamster cell lines has a pronounced effect in suppressing UV-induced mutagenesis [73]. Since the vast majority of UV-induced lesions are pyrimidine dimers, mutations occur at sites of adjacent pyrimidines. Expression of UV-DDB led to a marked decrease in mutations originating from lesions on the non-transcribed strand of the *HPRT* gene. By contrast, there was no detectable effect on mutations originating from the transcribed strand. This result was consistent with the role of UV-DDB in repairing non-transcribed DNA. Unrepaired CPDs generate mutations, since replication past these lesions is performed by bypass polymerases, which have severely decreased fidelity [49].

The observation that UV-DDB suppresses mutagenesis without affecting UV sensitivity demonstrates that cell survival and mutagenesis can be dissociated from each other. CS provides another example of this dissociation. CS patients exhibit profound UV-sensitivity without a significantly increased risk for skin cancer [53]. GGR remains intact in CS cells and this repair activity appears to be enough to suppress mutagenesis. On the other hand, the disruption of TCR in CS cells is sufficient to cause severe UV-sensitivity. Perhaps stalling of RNA polymerase II at unrepaired DNA lesions produces a signal for apoptosis [46].

Although rodent models of human cancer have been widely used, the deficiency of DDB2 and GGR in rodents presents a significant limitation. For example, the CSB knockout mouse fails to recapitulate the human disease, since the CS mouse is highly susceptible to skin cancer, while CS individuals are not [77]. This occurs because CSB knockout mice lose TCR in the setting of deficient GGR of CPDs, while CSB deficient humans lose TCR, but retain enough repair capacity via GGR to suppress skin cancer. Rodent models for cancer can be improved by engineering the regulated expression of *DDB2* in mice to confer GGR of CPDs similar to the levels seen in humans.

9. Cell free systems for NER

A number of investigators have succeeded in observing NER in vitro using either cell free extracts or reconstitution with highly purified proteins [1]. There is general agreement that dual incision of damaged DNA in cell free systems requires the activity of 15 to 18 polypeptides. The dual incision factors are the XPA protein, the heterotrimeric single-stranded DNA-binding protein RPA, the XPC/HR23B heterodimer, the six to nine subunit transcription factor TFIIH (which includes the XPB and XPD proteins), XPG nuclease and ERCC1/XPF nuclease.

XPA and RPA appear to act together in recognizing DNA damage. In a pull-down assay, the combination of XPA and RPA is more effective in discriminating between damaged and undamaged DNA than either XPA or RPA alone [19,40]. The XPC protein is tightly associated in cells with HR23B or, less often, with HR23A [68]. In repair synthesis assays with UV-damaged plasmid, the repair activity of XPC was stimulated two to four-fold by the addition of either HR23B or HR23A, suggesting some functional redundancy between the two proteins.

In exploring the activity of UV-DDB in cell free systems, different investigators have come to apparently contradictory conclusions. However, recent results suggest that the contradictions can now be resolved. To understand these experiments, it is critical to take note of the DNA substrate. Substrates have varied in length, the presence or absence of undamaged competitor

DNA and in the type of DNA lesion. DNA damage has been introduced at random by UV irradiation or incorporated into a synthesized oligonucleotide as a single CPD, 6–4 photoproduct, cisplatin adduct or cholesterol adduct. Interpretation of the results requires attention to the fact that different lesions may be recognized by different proteins. For example, data from intact cells demonstrate that UV-DDB is required for GGR of CPDs and plays an accessory role in GGR of 6–4 photoproducts; XPC is also required for GGR of both lesions; and XPA is required for GGR and TCR of both lesions. We will focus on the results of in vitro experiments with 6–4 photoproducts and CPDs because the repair of those lesions has been well studied with in vivo experiments.

10. UV-DDB directs the repair of CPDs in vitro

After Tang et al. reported that the expression of UV-DDB conferred GGR of CPDs to intact hamster cells [73], Wakasugi et al. investigated the effect of adding purified UV-DDB to hamster cell extracts [82]. For a DNA substrate containing a single CPD, UV-DDB stimulated a three to four-fold increase in nucleotide excision. Moreover, the addition of XPA protein or XPA protein plus RPA further enhanced excision in the presence of UV-DDB up to 7- and 10-fold, respectively. An EMSA demonstrated the formation of a complex of UV-DDB, XPA protein and RPA at the site of the CPD lesion, suggesting that UV-DDB may recruit XPA and RPA to the lesion.

11. Role of UV-DDB in the repair of 6–4 photoproducts in vitro

Kazantsev et al. reported that extracts prepared from XP-E cells were severely defective in excising 6–4 photoproducts [31]. Surprisingly, excision activity was restored by addition of purified RPA, but not UV-DDB. Otrin et al. revisited these puzzling results using extracts from three newly identified XP-E cell lines lacking UV-DDB [59]. These XP-E extracts did not display a significant defect in NER with either UV-irradiated DNA or a cisplatin lesion as substrate. NER was stimulated by the addition of RPA but not UV-DDB, but the RPA effect was nonspecific, since it occurred in extracts from XP-E, normal or complemented extracts from other XP cells. The XP-E cells used by Kazantsev et al. grow poorly and may not yield enough cells to make reliably active extracts. RPA is present at normal levels and is not mutated in XP-E cells. Thus, rescue of XP-E extracts by RPA as reported by Kazantsev et al. may be due to a non-specific effect of RPA on inactive extracts.

When NER of 6–4 photoproducts was reconstituted from purified recombinant proteins, the addition of UV-DDB had no effect and even inhibited excision under conditions in which XPA and RPA were rate limiting [31]. For a DNA substrate containing a single 6–4 photoproduct, Wakasugi et al. showed that addition of high concentrations of UV-DDB to the hamster extracts inhibited nucleotide excision, although adding XPA protein and RPA reversed the inhibition [82]. More recently, Wakasugi et al. showed that low concentrations of UV-DDB stimulated the excision of 6–4 photoproducts by two-fold [80].

UV-DDB binds with high affinity to 6–4 photoproducts, but cell free systems may not support its subsequent dissociation. In a plausible model, UV-DDB recognizes the DNA lesion, recruits one or more other repair proteins and then dissociates from the lesion to permit full assembly of the core NER complex. Dissociation of UV-DDB may be mediated by post-translational modification. For example, the DDB2 subunit of UV-DDB associates with Cullin 4A [66], which targets DDB2 for ubiquitination and proteosomal degradation [52]. Proteosomal degradation is not included in the reconstituted system of Wakasugi et al. Perhaps, high concentrations of UV-DDB inhibit excision of 6–4 photoproducts because excess UV-DDB is not removed and interferes with the activity of repair proteins recruited to the lesion by UV-DDB.

12. How is damaged DNA targeted for NER?

Although NER system is capable of recognizing many forms of DNA damage, it has been difficult to understand how recognition occurs. UV-DDB, XPC/HR23B, XPA, RPA and TFIIH all display some preference for binding to damaged over undamaged DNA [86]. However, there has been some debate on which protein is critical for the initial step of damage recognition.

Recognition of DNA damage appears to occur by different mechanisms in TCR and GGR. In TCR, which usually occurs more rapidly than GGR, the initial step of damage recognition is believed to occur when RNA polymerase II stalls at the site of the lesion and then recruits additional repair factors (Fig. 2) [17]. In GGR, damage recognition must occur independently of RNA polymerase II. UV-DDB and XPC/HR23B are required only for GGR, while XPA, RPA and TFIIH are required for both TCR and GGR. Investigators have focused their attention on which of these proteins is involved in the initial step of damage recognition for GGR.

There has been conflicting evidence for the order in which XPA/RPA and XPC/HR23B act. Sugasawa et al. reported that purified XPC/HR23B exhibited a strong preference for damaged DNA [67]. Pre-incubation of damaged DNA with XPC/HR23B followed by the addition of other repair factors resulted in a higher rate of repair than was observed with pre-incubation of damaged DNA with XPA/RPA. Sugasawa et al. concluded that XPC/HR23B acts before XPA/RPA during GGR. In direct contrast, Wakasugi and Sancar detected only a slight preference of XPC/HR23B for damaged DNA [81]. The rate of damage removal was five-fold faster when damaged DNA was pre-incubated with XPA/RPA rather than with XPC/HR23B.

To resolve the discrepancy in binding to damaged DNA, Batty et al. re-examined the binding properties of XPC/HR23B, confirming a strong preference for binding to damaged DNA [3]. They conclude that the most important sources of discrepancy were the use of short DNA probes (49–60 bp) and the absence of competitor DNA in the experiments of Wakasugi and Sancar. In the absence of competitor DNA, the discrimination of XPC/HR23B for damaged DNA might not be fully realized. With a short length DNA probe, binding of XPC/HR23B might not be optimal. By contrast, Batty et al. incubated a 136 bp DNA probe and obtained a distinct bandshift after adding an excess of undamaged competitor DNA. Thus, the use of a longer DNA probe and the addition of competitor DNA, conditions that are likely to be more relevant to DNA repair in vivo, indicated that XPC/HR23B acts before XPA/RPA [3,69].

Batty et al. compared the binding properties of UV-DDB and XPC/HR23B, measuring both molar affinity for damaged DNA and relative discrimination between damaged and undamaged DNA [3]. The molar affinity of UV-DDB is about 350-fold higher than the molar affinity of XPC/HR23B for UV-damaged DNA. This high molar affinity for damaged DNA explains why UV-DDB is the only binding activity that can be detected by an EMSA in crude extracts. Extracts of XP-E cells and hamster cells, which lack UV-DDB, show no binding activity that can be attributed to XPC/HR23B or XPA/RPA. On the other hand, UV-DDB and XPC/HR23B show roughly equivalent discrimination for 6–4 photoproducts over undamaged DNA.

Purified UV-DDB and XPC/HR23B form distinct complexes in an EMSA with a 6–4 photoproduct containing DNA probe [3]. A mixture of UV-DDB and XPC/HR23B gave two independent shifted complexes of the same mobility as seen for the individual proteins. No new bandshifts were observed that would be diagnostic of a co-complex of the two proteins with a single molecule of DNA, and there was no sign that XPC/HR23B is capable of displacing UV-DDB from the DNA in this purified system. These results are consistent with the hypothesis that XPC can act independently of UV-DDB in targeting 6–4 photoproducts for repair (Fig. 2). It is notable that UV-DDB produced much more binding activity than an equimolar amount of XPC/HR23B, consistent with its higher molar affinity for damaged DNA. Despite its higher affinity for 6–4 damage, UV-DDB is not essential for recruiting the core

NER proteins, since 6–4 repair is only mildly impaired in XP-E cells and severely impaired in XPC cells. This mild impairment in XP-E suggest that UV-DDB may facilitate XPC binding to 6–4 photoproducts.

In contrast to the repair of 6–4 photoproducts, the GGR repair of CPDs is much less efficient [51] because CPDs produce only a mild distortion in the DNA structure. UV-DDB binds with a low affinity to CPDs that is detectable by EMSA [15,21,64]. XPA fails to bind CPDs in an EMSA [28], although weak binding is detectable by surface plasmon resonance [83]. XPC/HR23B binding to CPDs is too weak to detect in an EMSA [37]. Interestingly, XPC/HR23B binding becomes significant when a mismatched G·G is inserted opposite the T·T CPD to produce increased distortion in the DNA. In addition, XPC/HR23B binds to undamaged DNA substrates containing a bubble of three to five mismatches, suggesting that it recognizes damage by sensing distortion in the helix rather than the lesion itself [69]. However, the presence of a lesion is required for the subsequent incision step, suggesting that the other repair proteins subsequently recruited to the damage site provide a damage verification function.

The binding of UV-DDB to damaged DNA induces a major distortion, bending the DNA by an angle of 55° [15]. This leads us to hypothesize that UV-DDB binds to the CPD and distorts the DNA helix so that XPC/HR23B can be recruited to the CPD site (Fig. 2). XPC/HR23B promotes stable binding of XPA/RPA to UV-irradiated DNA [41]. Subsequent recruitment of TFIIH to the lesion is promoted by XPA/RPA [57,60] and/or by XPC/HR23B [41,87]. XPA/RPA and/or TFIIH may verify the presence of the damage and then direct excision of the damaged oligonucleotide by the ERCC1/XPF and XPG endonucleases.

13. Regulation of UV-DDB

In response to DNA damage, the p53 protein is phosphorylated and becomes resistant to proteosomal degradation. Increased p53 levels stimulate the transcription of genes involved in cell cycle arrest and apoptosis. In addition, p53 enhances transcription of the *DDB2* gene, which then produces an increase in GGR of CPDs [22,73].

Basal levels of *DDB2* transcription are dependent on the presence of p53 protein even in the absence of DNA damage. Transcription of *DDB2* increases further after DNA damage. Levels of *DDB2* mRNA increase over 24 h after UV exposure and over 4 h after IR exposure, time courses that mirror the accumulation of p53 [22]. In the absence of p53, the basal levels of *DDB2* mRNA are severely reduced and do not increase after either UV or IR [22]. Furthermore, forced expression of p53 with a tetracycline-regulated promoter induces transcription of *DDB2* and increased levels of UV-DDB. By contrast, *DDB1* transcription does not depend on p53 and is unaffected by exposure of cells to UV or IR.

The transcriptional response of *DDB2* to DNA damage is mediated by a consensus binding site for p53 in the 5′ untranslated region of the *DDB2* gene [72]. This region will bind p53 protein in an EMSA and will drive the expression of a reporter construct when co-transfected with a p53 expression vector.

The subcellular locations of DDB1 and DDB2 are regulated in response to DNA damage. In undamaged wild-type and XP-E cells, the DDB2 protein localizes to the nucleus, while DDB1 is found in both the cytoplasm and the nucleus [44,58,65]. Following UV exposure, DDB1 redistributes to the nucleus. It appears that DDB2 plays a role in transporting DDB1 to the nucleus, because expression of *DDB2* with non-sense mutations from XP-E cells reduces the UV-induced nuclear accumulation of DDB1 [44].

UV-DDB appears to be regulated post-translationally by phosphorylation. Treatment of purified UV-DDB with alkaline phosphatase reduces binding activity (Hwang and Chu,

unpublished results). A DDB1 peptide was found to interact with the c-*abl* tyrosine kinase in a yeast two hybrid screen [7]. Co-precipitation experiments demonstrated that c-*abl* forms a stable complex with the DDB1/DDB2 heterodimer. Transfection of a c-*abl* expression vector produced a decline in UV-DDB as measured by EMSA and transfection of a dominant negative mutant of c-*abl* greatly enhanced UV-DDB. In addition, c-*abl* expression led to increased tyrosine phosphorylation of DDB2. These results suggest that c-*abl* modifies DDB2 post-translationally by tyrosine phosphorylation, leading to decreased binding of UV-DDB to UV-damaged DNA.

Several DNA damaging agents, including IR, cisplatin and mitomycin C, but not UV, activate the c-*abl* tyrosine kinase [35,45]. Thus, IR appears to lead to c-*abl*-dependent modification of DDB2 and decreased binding activity in the face of increased DDB2 transcription.

To explain these apparently paradoxical effects on DDB2, we hypothesize that the c-*abl* tyrosine kinase remodels UV-DDB so that binding to UV-damaged DNA is suppressed in favor of increased binding to IR-induced lesions. It is noteworthy that a genome-wide search for genes induced by ionizing radiation yielded several NER genes: *DDB2*, *XPC*, *gadd45* and *PCNA* [75]. Furthermore, there have been reports of a novel form of excision repair after IR [12,38]. This repair pathway might include DDB2, XPC, gadd45 and PCNA. In addition, the protein purified by Feldberg and Grossman, which is likely to be identical to UV-DDB, also bound to DNA damaged by ionizing radiation and an enzymatic superoxide-generating system [11]. Although tyrosine phosphorylation of DDB2 modifies UV-DDB so that it binds less effectively to UV-damaged DNA, the phosphorylated protein may then bind with higher affinity to forms of base damage produced by ionizing radiation, targeting those lesions for excision repair.

14. Possible roles for UV-DDB in transcription, cell cycle and other pathways

The DDB2 protein interacts with transcription factor E2F1 and, in conjunction with DDB1, enhances the transcriptional activity of E2F1 [18]. Expression of UV-DDB reversed the inhibition of E2F1 by the retinoblastoma protein. Mutant forms of DDB2 found in XP-E patients associated with E2F1 as efficiently as wild-type DDB2, but were severely impaired in stimulating E2F1-activated transcription [65]. Although these experiments are based on transfection and forced expression of DDB2, DDB1 and E2F1, the data suggest that UV-DDB may function as a transcriptional partner of E2F1. Shiyanov et al. speculate that when cells are exposed to DNA damage, UV-DDB binds to the damaged DNA and is sequestered from E2F1. This retards the cell cycle by down-regulating E2F1-activated genes, which are involved in DNA replication and S-phase entry. In co-transfection experiments, mutant forms of DDB2 from XP-E patients are severely impaired in stimulating E2F1-activated transcription [65]. If this observation is physiologically relevant, the resultant down-regulation in cell cycle entry cannot be severe, since XP-E patients do not exhibit generalized growth and developmental abnormalities and XP-E cells can grow in culture.

The DDB2 subunit associates with Cullin 4A, raising another possible link to cell cycle regulation [66]. Cullin 4A is a member of the Cullin family of ubiquitin-protein E3 ligases responsible for degrading cyclins A and E. Degradation of DDB2 might contribute to cell cycle arrest by preventing its enhancement of E2F1 transcriptional activity.

Like DDB2, the DDB1 protein may have activities unrelated to DNA repair. The DDB1 protein binds as a monomer or homodimer to a liver specific regulatory sequence in the *apoB* gene [36]. Moreover, antibodies raised against a peptide derived from DDB1 inhibited apoB transcription in vitro.

The DDB1 protein interacts with several viral proteins, such as the V protein from paramyxovirus simian virus 5 (SV5), as well as the V proteins of mumps virus, human parainfluenza virus type 2 and measles virus [42,43]. Infection of cells with SV5 or transfection with V protein will slow cell cycle progression. The V protein C terminal domain, which interacts with DDB1, is required for this activity. The cell cycle slowing can be partially restored by co-expression of DDB1. The DDB1 protein also interacts with the hepatitis B virus X (HBx) protein, which is required for virus multiplication in vivo [39]. HBx transactivates a wide variety of viral and cellular promoters, raising the possibility that the previously described transcriptional activity of DDB1 might play a role in HBx transactivation. A panel of HBx mutant proteins, some of which no longer bind to DDB1, was used to test this hypothesis [85]. The binding of HBx mutant proteins to DDB1 was generally but not completely correlated with transactivation. Expression of HBx protein inhibits cellular DNA repair and binding of HBx to DDB1 was generally but not completely correlated with inhibition of DNA repair [4]. HBx also interacts with the p53 protein- and inhibition of DNA repair is p53-dependent [63]. Thus, the inhibition of DNA repair by HBx could be mediated by its interaction with DDB1 or p53 or both proteins.

The DDB1 subunit also interacts with the cytoplasmic domain of the Alzheimer's amyloid precursor protein (APP) [84]. Abnormal metabolism of APP may lead to overproduction of amyloid β-protein and thus contribute to the etiology of Alzheimer's disease. Lower DNA repair activity has been reported in cells from Alzheimer's disease patients and this could contribute to neuronal degeneration.

In summary, UV-DDB has potential roles in regulating transcription or the cell cycle or in the pathogenesis of viral infections or neuronal degeneration in Alzheimer's disease. These roles have usually been studied in the context of forced expression from transfected DNA vectors, which may not accurately reflect true cellular physiology. More reliable conclusions could be drawn from cells in which either the *DDB1* or *DDB2* gene has been knocked out, but such cells have not yet been constructed.

15. Conclusions

A role for UV-DDB in the GGR of CPDs has been established in vivo and in vitro [73,82]. In addition, UV-DDB appears to play an accessory role in the GGR of 6–4 photoproducts from observations of intact XP-E cells [22,26], but this activity has not been observed in vitro. Several proteins have damage specific binding activity, including UV-DDB, XPC/HR23B, XPA/RPA and TFIIH. The order of assembly of these proteins remains unknown, although there is evidence in the case of CPDs that binding of UV-DDB initiates the process. A postulated role for UV-DDB in chromatin remodeling remains to be proven. It is not yet known how UV-DDB is removed from the site of the lesion after it has recruited other DNA repair proteins.

The DDB2 subunit appears to be critical for the regulation of UV-DDB. The DDB2 protein is rate limiting for the activation of binding activity [24]. The *DDB2* gene is transcriptionally activated by p53 [22] and the DDB2 protein is modified by phosphorylation [7] and ubiquitination [52]. This intricate dance of regulatory proteins suggests that UV-DDB plays a multifaceted role against the assault of genotoxic agents. Nevertheless, it is still not known how DDB2 activates DDB1 for DNA-binding. More work must be done to test hypotheses that UV-DDB can be remodeled to recognize different types of DNA lesions.

The *DDB1* gene is strongly conserved among a diverse spectrum of eukaryotes, but there is no such conservation for *DDB2*. It is possible that DDB1 has an ancient function not related to DNA repair. Evidence for such functions comes from experiments suggesting that UV-DDB

can activate transcription by its association with E2F1 or hepatitis virus X protein [18,39]. Further work is required to establish the physiological relevance of these activities. Perhaps DDB2 is the product of a recent evolutionary adaptation in vertebrates to increase the efficiency of DNA repair. It is interesting that p53 and DDB2 have roles in maintaining genomic stability to prevent cancer, and both are not present in unicellular eukaryotes.

In summary, DDB1 and DDB2 participate in a complex cellular dance with many protein partners. The dance includes a carefully orchestrated response to DNA damage with functions in DNA repair, and possibly cell cycle arrest. Other functions are also emerging as we listen to the music and identify the partners.

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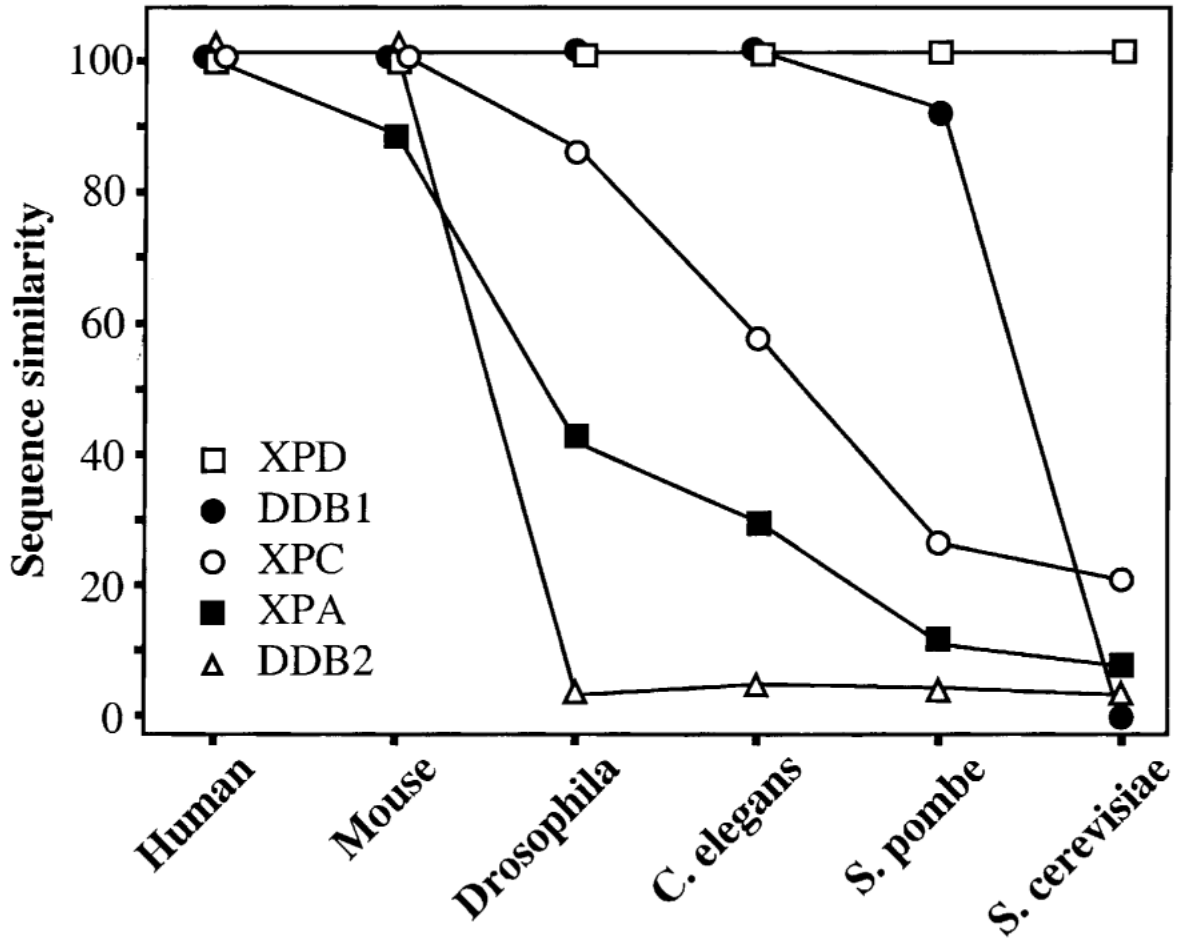

Fig. 1.

Similarity of human DNA repair proteins to proteins from other species. The BLAST 2.0 algorithm was used to identify amino acid sequences in mouse, *Drosophila*, *C. elegans*, *S. pombe* and *S. cerevisiae* that were similar to XPA, XPC, XPD, DDB1 or DDB2 in humans. The BLAST algorithm calculates the significance of sequence similarity within a local alignment and provides an expectation value (E) as a parameter that describes the number of matches expected by chance when searching the BLAST database. For example, the XPD protein is highly similar in human and in yeast with an *E* value of e^{-105}. This means that there is a probability of e^{-105} that this sequence similarity occurred by chance when searching the entire database. To represent this value graphically, ln(1/*E*) was calculated as a measure "sequence similarity" between the human protein and its homolog in other organisms. Sequence similarity values greater than 100 have such a high significance that they were reset to a cap of 100 in order to better display sequences with lower sequence similarity values.

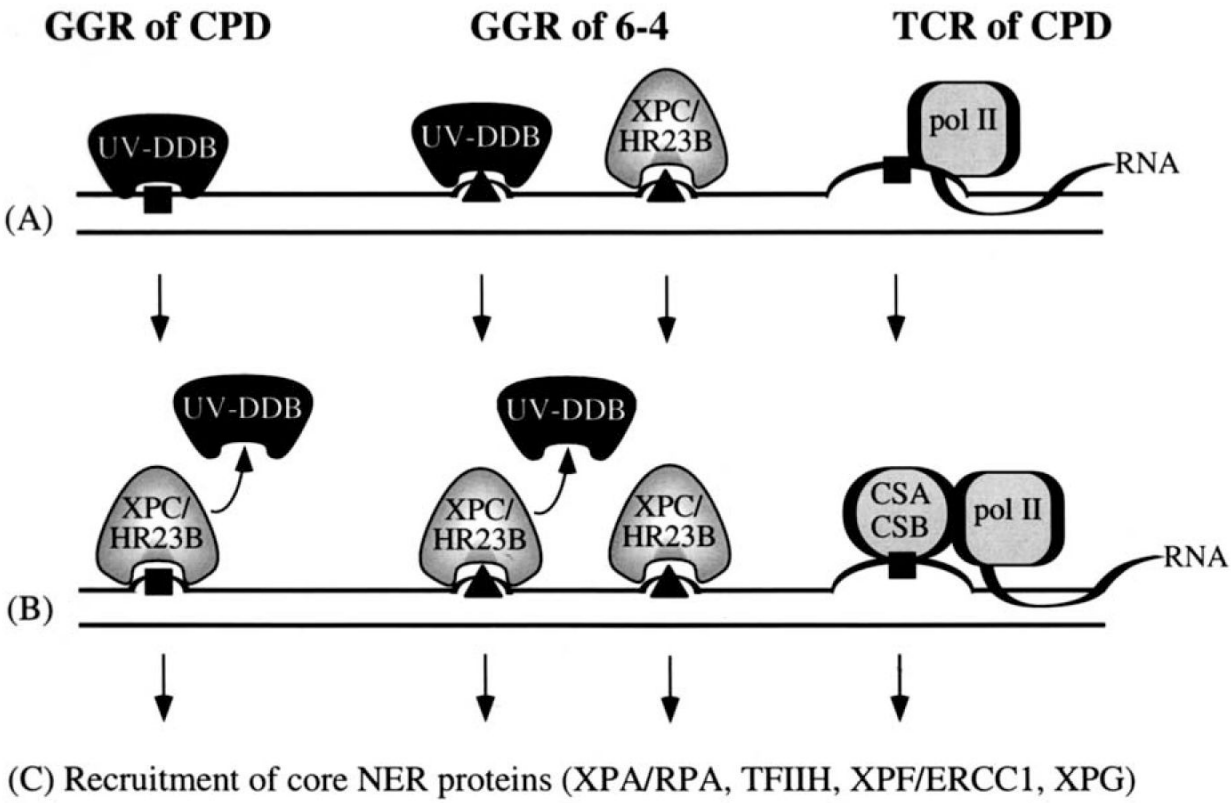

Fig. 2.

Model for how lesions are targeted for NER. (A) Damage recognition. UV induces DNA damage in the form of a CPD (■) or 6–4 photoproduct (▲). Damage recognition for GGR occurs by binding of UV-DDB to the CPD, or binding of UV-DDB or XPC/HR23B to the 6–4 photoproduct. Damage recognition for TCR occurs by stalling of RNA polymerase II (pol II) at the site of the lesion. (B) Preparation for coupling to core NER proteins. Binding of UV-DDB to the CPD for GGR induces sufficient distortion in the DNA helix to permit recruitment of XPC/HR23B. The mechanism for removing UV-DDB is not known, but may occur by displacement or proteosomal degradation. XPC/HR23B also replaces UV-DDB at the 6–4 photoproduct. The stalled pol II recruits CSA/CSB for TCR. (C) Recruitment of core NER proteins. Core repair proteins are assembled at the site for lesion verification and DNA unwinding (XPA/RPA and TFIIH) followed by dual incision 3′ and then 5′ to the lesion (XPG and ERCC1/XPF, respectively).

Table 1

Individuals originally assigned to XP-E

Number	Cell line	Age/gender/ethnicity	Skin cancer	UV-DDB	Syndrome	DDB2	DDB1
1	XP2RO	16/F/Dutch	BCC	−	XP-E	R273H	WT
2	XP3RO	29/F/Dutch	BCC	−	XP-E	R273H	WT
3	XP24KO	24/F/Japanese	None	+	UV^{S}S	ND	ND
4	XP26KO	8/M/Japanese	None	+	XP-E or UV^{S}S	ND	ND
5	XP43TO	30/F/Japanese	BCC	+	XP-V	ND	ND
6	XP70TO	5/F/Japanese	None	+	UV^{S}S	ND	ND
7	XP80TO	50/F/Japanese	BCC	+	UV^{S}S	ND	ND
8	XP81TO	42/F/Japanese	BCC	+	UV^{S}S	ND	ND
9	XP82TO	41/F/Japanese	None	−	XP-E	K244E	WT
10	XP89TO	15/F/Japanese	None	+	XP-F	WT	WT
11	XP93TO	8/F/Japanese	ND	+	XP-E	WT	WT
12	XP95TO	ND/ND/Japanese	ND	+	XP-E	WT	WT
13	XP23PV	ND/F/Italian	ND	−	XP-E	ND	ND
14	XP25PV	ND/F/Italian	ND	−	XP-E	ND	ND
15	GM01389	21/F/Caucasian	ND	−	XP-E	L350P/del349	WT
16	Ops	62/F/Japanese	MM/BCC/SCC	−	XP-E	R313ter	WT

Data are summarized for individuals originally reported to belong to XP complementation group E. Most individuals suffered from multiple skin cancers, including basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (MM). Several individuals were subsequently reclassified as XP variant (XP-V), XP group F (XP-F) or UV-sensitive syndrome (UV^{S}S). Abbreviations: ND, not determined; WT, wild-type.

Table 2

Lesions recognized by UV-DDB

Lesions	UV-DDB binding	References
6–4 Photoproduct	+++	[15,62,64]
Trans,*syn*-CPD	++	[64]
Abasic site	++	[15,62]
Cis,*syn*-CPD	+	[15,62]
Cisplatin adduct	+	[15,62]
Nitrogen mustard adduct	+	[62]
Single-stranded DNA	+	[62]
4-Nitroquinolone oxide adduct	+	[62]
Methylcytosine	−	[62]
Nicked DNA	−	[15]
Psoralen adduct	−	[62,64]
Transplatin adduct	−	[62]
T·C or T·T mismatch	−	[15]
^{6}O-Methylguanine	−	[15]
8-Oxo-guanine	−	[15]