

Nucleotide excision repair and human syndromes

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DNA damage is implicated in cancer and aging, and several DNA repair mechanisms exist that safeguard the genome from these deleterious consequences. Nucleotide excision repair (NER) removes a wide diversity of lesions, the main of which include UV-induced lesions, bulky chemical adducts and some forms of oxidative damage. The NER process involves the action of at least 30 proteins in a ‘cut-and-paste’-like mechanism. The consequences of a defect in one of the NER proteins are apparent from three rare recessive syndromes: xeroderma pigmentosum (XP), Cockayne syndrome (CS) and the photosensitive form of the brittle hair disorder trichothiodystrophy (TTD). Sun-sensitive skin is associated with skin cancer predisposition in the case of XP, but remarkably not in CS and TTD. Moreover, the spectrum of clinical symptoms differs considerably between the three syndromes. CS and TTD patients exhibit a spectrum of neurodevelopmental abnormalities and, in addition, TTD is associated with ichthyosis and brittle hair. These typical CS and TTD abnormalities are difficult to comprehend as a consequence of defective NER. This review briefly describes the biochemistry of the NER process, summarizes the clinical features of the NER disorders and speculates on the molecular basis underlying these pleiotropic syndromes.

DNA damage and its consequences

DNA contains the blueprint for the proper development, functioning and reproduction of every organism. Alterations affecting the structure and integrity of DNA molecules can arise spontaneously through intrinsic instability of chemical bonds in DNA (e.g. deamination, depurination, etc.) or can be induced by chemical compounds and irradiation. DNA lesions are of many different types (Figure 1) including single- and double-strand breaks (induced by X-rays), inter- and intrastrand crosslinks (caused by chemical agents, such as the cytostatic cisplatin) and different kinds of base modifications. At the cellular level, DNA lesions hamper processes like transcription and replication resulting in cell-cycle arrest, (programmed)

Abbreviations: 6-4PPs, pyrimidine (6-4) pyrimidone photoproducts; CAK, cdk-activating kinase; CPDs, *cis-syn*-cyclobutane pyrimidine dimers; CRP, cysteine-rich matrix proteins; CS, Cockayne syndrome; GG-NER, global genome nucleotide excision repair; IF, intermediate keratin filaments; NER, nucleotide excision repair; RPA, replication protein A; TC-NER, transcription-coupled repair; TTD, trichothiodystrophy; UDS, unscheduled DNA synthesis; XP, xeroderma pigmentosum.

cell death and genomic instability (mutagenesis). At the organismal level, DNA lesions have been implicated in several distinct genetically inherited diseases, in carcinogenesis, origin of genetic disorders and in ageing. A clear example of the deleterious effects of genotoxic agents in man is the strong correlation between UV (sunlight) exposure or smoking cigarettes and the development of skin and lung cancer, respectively (1). Both sunlight and cigarette smoke are exogenous sources of DNA damage. DNA base modifications can also arise endogenously through cellular metabolites; for instance, oxidative DNA damage can result from free radicals generated as a by-product of active oxidative metabolism. Accumulating evidence suggests that modulation of oxidative stress plays a role in ageing (2).

To prevent the detrimental consequences of DNA damage a complex network of complementary DNA repair mechanisms has arisen (Figure 1). Briefly, double-strand breaks are repaired by homologous recombination-dependent repair or in an end-joining reaction, and most small base modifications are removed by base excision repair (BER). Nucleotide excision repair (NER) removes primarily bulky, helix-distorting adducts. However, considerable overlap exists in substrate specificity of repair pathways, and certain proteins are used in more than one pathway (3). Here we will focus on recent progress made in understanding the molecular mechanism and biological impact of the versatile NER system. A comprehensive overview of different DNA repair pathways, including mutagenesis has been given previously (4).

Nucleotide excision repair

Elucidation of the core mechanism of NER in *Escherichia coli* served as a paradigm for studies on NER in other organisms. The basic principle in prokaryotes is removal of a small single-stranded segment of 12–13 nucleotides containing the lesion by dual incision of the damaged strand. Gap-filling repair synthesis occurs using the intact complementary strand as template. The general principle is evolutionarily conserved from bacteria to man but the proteins involved share little homology over the prokaryotic to eukaryotic border and many steps in the mammalian system are more complex. Most mammalian NER genes have been identified now and, recently, the mammalian NER reaction was reconstituted *in vitro* using purified proteins (5). This review summarizes the substrate specificity and the distinct steps of mammalian NER and human NER syndromes. The biochemistry of NER has been described in more detail previously (6–8).

Lesions removed by NER

NER is the most flexible of all DNA repair mechanisms because of its ability to eliminate a plethora of structurally unrelated DNA lesions. The common denominator of the different types of damage induced by the numerous chemicals to which NER-deficient cells are sensitive seems to be the generation of bulky base adducts which cause significant helical distortion in addition to a change in the DNA chemistry (8,9).

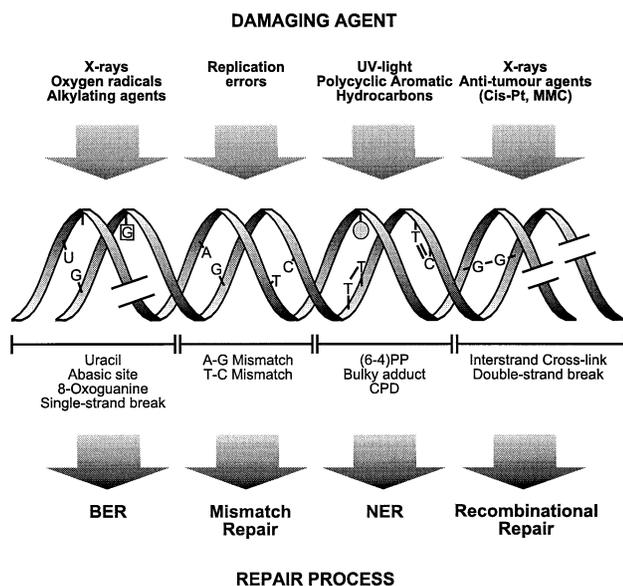


Fig. 1. DNA lesions and repair mechanisms. (Top) Common DNA damaging agents. (Middle) Examples of lesions that can be introduced by these agents into the DNA double helix. (Bottom) The most frequently used repair mechanisms for such lesions. Not depicted but important to realize is that distinct damaging sources can induce similar types of DNA lesions, that one agent in general induces more than one type of damage and that also the lesion spectrum of different repair pathways may overlap. Adapted from (6). For abbreviations see text.

The clinically most relevant NER substrates are *cis-syn*-cyclobutane dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs). Both are formed between adjacent pyrimidines, and they constitute the two major classes of lesions induced by solar UV light. The more helix-distorting 6-4PPs are repaired 5-fold faster than CPDs and, although CPDs are more abundant, UV hypersensitivity of rodent and human cell lines correlates better with the capacity to excise 6-4PPs than with CPD removal from the genome (10). Other NER substrates include bulky chemical adducts such as large polycyclic aromatic hydrocarbons (induced by compounds in cigarette smoke) and the particularly distorting interstrand crosslinks, induced by chemotherapeutic agents such as cisplatin and probably also by certain cellular metabolites (11). NER has also been implicated in removal of minor base damage induced by alkylating and oxidizing agents which are generally not helix distorting (4). BER is considered as the main pathway involved in repair of these types of damage, but NER may be considered as a back-up system (12,13). The relative importance of NER in repair of alkyl and oxidative damage has not been firmly established yet. However, its involvement emphasizes the versatility of the NER mechanism, which also explains how various endogenous and exogenous genotoxic agents may contribute to the large spectrum of clinical symptoms associated with defects in one of the NER components.

The NER mechanism

The NER process involves the action of about 20–30 proteins in successive steps of damage recognition, local opening of the DNA double helix around the injury, and incision of the damaged strand on either side of the lesion. After excision of the damage-containing oligonucleotide the resulting gap is filled by DNA repair synthesis, followed by strand ligation. A

(still in part speculative) model for NER compiling results obtained by many groups is presented below and depicted in Figure 2.

Damage recognition

Recent findings have established that binding of the XPC/HHR23B complex is the initial, damage-recognizing step in NER (14), recruiting the entire repair protein apparatus to the injury. Such a scenario is supported by indications for an early role of XPC in the repair reaction (15,16). Since locally premelted DNA bypasses the need for XPC/hHR23B (17), the complex may, in addition to lesion detection, increase single-strandedness at the site of the DNA injury. An auxiliary role for the XPE protein in damage recognition of cyclobutane pyrimidine lesions has been proposed, because it has affinity for UV-damaged DNA (18). Also XPA displays a high affinity for injured DNA (19), especially in a single-stranded context (9). As discussed below this protein may be important for verification of the damage and for properly organizing the repair apparatus around the lesion.

Lesion demarcation

The next step is the formation of an open complex, requiring a local unwinding of the DNA helix and demarcation of the lesion. XPA has many interactions with other NER components, for instance with the single-strand binding complex replication protein A (RPA) (20), the TFIIH complex (21) and the ERCC1/XPF endonuclease (22), in addition to affinity for a variety of lesions particularly when base pairing is disrupted (9). Hence, XPA may orchestrate the repair machinery around the DNA lesion when XPC/HHR23B with or without the help of TFIIH has locally opened the helix. Full opening of the DNA helix around the lesion is dependent on the presence of ATP (15), strongly arguing that the helicases of the TFIIH complex (discussed below) are actively involved. DNA unwinding by TFIIH may be facilitated by RPA, a heterotrimeric complex involved in NER, replication and recombination (23). In NER it probably binds to the single-stranded region of the undamaged strand with defined polarity (24). The optimal binding patch of RPA is 30 nucleotides (25), which is similar to the size of the fully opened repair complex and the size of the released damage-containing oligonucleotide (26).

The role of TFIIH in NER and basal transcription

TFIIH is a protein complex of nine subunits, which was originally identified as an essential factor in basal transcription initiation (27). The p89 and p80 subunits were identified as the previously discovered XPB and XPD NER helicases (28,29), which contain, respectively, ATPase-driven 3'→5' and 5'→3' directed DNA unwinding activity. TFIIH is required for local unwinding of the DNA helix around the lesion in NER (15) and in the transcription initiation of RNA polymerase II at the promoter (30) (Figure 2). In accordance with an essential role in basal transcription, mice with inactivating mutations in the TFIIH subunits XPB and XPD are inviable (31; G.Weeda, unpublished data). Other TFIIH components include Cdk7, cyclin H and MAT1, constituting the cdk-activating kinase (CAK) complex associated with TFIIH. The CAK complex is able to phosphorylate cyclin-dependent kinases (CDKs) involved in cell-cycle regulation, and it is required for phosphorylation of the C-terminal domain of RNA polymerase II (32). The CAK complex is more loosely associated with TFIIH and occurs in a free form as well. It is not required for NER *in vitro*, suggesting that TFIIH can be in either a transcription mode or a repair mode (33).

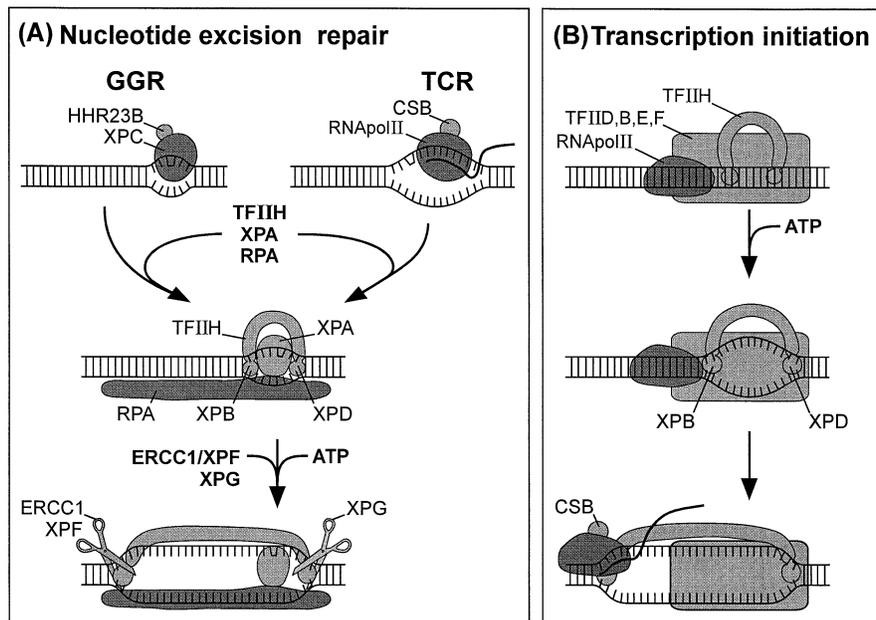


Fig. 2. Model for TC-NER, GG-NER and the role of TFIIH in transcription and repair. **(A)** Model for TC-NER and GG-NER. Recognition of DNA damage can occur either by the XPC/HHR23B complex (specific for GG-NER) or by RNA polymerase and the CSA and CSB proteins (specifically engaged in TC-NER). Subsequently, DNA around the lesion is opened by the concerted action of RPA, XPA and the bi-directional XPB/XPD helicase subunits of TFIIH. This allows incisions of the damaged strand on both sides of the injury by the repair endonucleases ERCC1/XPF and XPG, excision of the lesion-containing oligonucleotide and gap-filling DNA synthesis. **(B)** TFIIH in transcription initiation of RNA polymerase II. After assembly of the pre-initiation transcription complex, consisting of five basal transcription factors and RNA polymerase II, the promoter region is opened by the XPB and XPD helicases of TFIIH. This allows formation of the first phosphodiester bond, promoter escape of RNA polymerase and transcription elongation. Adapted from (86).

Dual incision

After local unwinding and demarcation of the lesion, an oligonucleotide of 24–32 nucleotides containing the lesion is excised. This requires the structure-specific endonuclease activities of XPG at the 3' side of the open complex (34). The ERCC1/XPF complex cuts at the single-strand to double-strand transition 5' of the damage (35). In principle, both XPG and ERCC1/XPF can also incise the undamaged strand, but specificity of ERCC1/XPF seems to be coordinated by RPA, which binds with defined polarity to the undamaged strand. Its 3'-oriented side stimulates ERCC1/XPF whereas the 5'-oriented side inhibits endonuclease activity of ERCC1/XPF in the undamaged strand (24). RPA and XPG interact directly, but RPA alone is not sufficient to endow strand specificity upon XPG. The strong interaction between XPG and TFIIH (36) suggests that TFIIH is involved in XPG positioning.

Gap-filling and ligation

The last step in the NER reaction, gap-filling of the excised patch by repair DNA synthesis, is used for assaying NER activity *in vitro* and *in vivo*. An *in vitro* reconstituted repair reaction showed that efficient repair synthesis occurs after addition of the mammalian DNA replication factors RPA, RFC, PCNA and DNA polymerase δ and ϵ (37). The NER reaction is completed by ligation of the newly synthesized DNA. DNA ligase I is a likely candidate for this reaction. Mutations in the corresponding gene can give rise to a UV-sensitive phenotype (38).

Two different pathways

The reaction mechanism described above is designated global genome nucleotide excision repair (GG-NER), which removes DNA damage from any place in the genome. In contrast, lesions in the transcribed strand of actively transcribed genes are preferentially repaired via the NER-subpathway transcrip-

tion-coupled repair (TC-NER) (39,40). Both processes are essentially the same except for the initial damage recognition step, which is performed by XPC/HHR23B in GG-NER. This is the only NER factor that is known to be dispensable for TC-NER (41) (Figure 2A). Instead, the stalled RNA polymerase II complex itself seems to be the damage recognition signal in TC-NER and attracts the core of the NER machinery (17). Because a stalled RNA polymerase II sterically hinders accessibility of NER proteins, it has to withdraw or dissociate from the lesion for repair to occur (42). Cells with a defect in the CSA or CSB genes are specifically defective in TC-NER; the CSA protein contains WD-repeats, which are thought to be involved in formation of multi protein complexes (43) and CSB is a member of the SWI/SNF family of DNA-dependent ATPases implicated in chromatin remodeling (44). An *in vivo* interaction between CSB and RNA polymerase II was found (45), suggesting that CSA and CSB are involved in processing of a stalled RNA polymerase complex. However, the precise role of CSA and CSB in the process of TC-NER remains unclear. Besides NER the Cockayne syndrome (CS) proteins may link other DNA repair systems to help a blocked RNA polymerase complex (46).

NER deficiency syndromes

The consequences of a defect in one of the NER proteins are apparent from three rare recessive photosensitive syndromes: xeroderma pigmentosum (XP), CS and the photosensitive form of the brittle hair disorder trichothiodystrophy (TTD). Cell-fusion experiments have led to the identification of seven complementation groups within the NER-deficient class of XP patients (designated XP-A to XP-G), two in CS (CS-A and CS-B), three in the category of patients with combined XP and CS (XP-B, XP-D and XP-G) and also three in TTD (XP-

B, XP-D and TTD-A). Each of these groups reflects a defect in a distinct gene. It is remarkable that different mutations in the *XPB*, *XPD* and *XPG* genes are associated with a specific clinical outcome: either XP, or XP/CS or TTD (for *XPB* and *XPD*). Importantly, sun-sensitive skin is associated with skin cancer predisposition in the case of XP, but not in CS and TTD. In addition, the spectrum of clinical symptoms differs considerably between the three syndromes (reviewed in ref. 47) and many of the peculiar abnormalities of CS and TTD are difficult to comprehend as a consequence of defective NER.

Xeroderma pigmentosum

Parchment skin (xeroderma) and freckles (pigmentosum) are the prominent cutaneous hallmarks of XP patients, which are strikingly limited to sun-exposed areas of the skin. In addition, sun exposure of XP patients generally results in progressive degenerative alterations of the skin and eyes (47). The mean age of onset of these symptoms is 2 years (48). Furthermore, XP is associated with a >1000-fold increased risk to develop skin cancers, which are also largely confined to sun-exposed areas like the face, neck, head and even the tip of the tongue. XP patients mainly develop basal cell carcinomas and squamous cell carcinomas, and less frequently melanomas. The mean age of onset of the first skin neoplasm is 8 years, which is nearly 50 years earlier compared with the general population (48). Many XP individuals die of neoplasia, reducing the life span by ~30 years. Moreover, XP patients have a 10- to 20-fold increased risk of developing several types of internal cancers under the age of 20 years (49). Considering the involvement of NER in repair of certain chemically induced DNA lesions, as well as in repair of lesions induced by cellular metabolites, either category of lesions may play a role in these internal neoplasms.

A fraction of XP patients (~18%) displays progressive neurologic abnormalities. The underlying condition seems to be primary neuronal degeneration with loss of neurons. At the severe end of the clinical spectrum are patients with DeSanctis–Cacchione syndrome with microcephaly, progressive mental deterioration, dwarfism and impaired sexual development (50). It appears that individuals who only have a partial NER defect, like XP-F and XP-C patients, tend not to develop neurologic symptoms at all or develop them later in life as compared with patients with more severe NER defects (e.g. XP-A) (47). A possible explanation for the onset of neurological abnormalities in XP patients is that defective DNA repair in nerve cells of endogenous (oxidative) NER lesions induces neuronal death (51).

As outlined above, XP is characterized by genetic heterogeneity. Consequently, heterogeneity in severity of the repair defect and of symptoms such as sun sensitivity and neuronal abnormalities is observed. Many of the XP-A, XP-B, XP-D and XP-G individuals exhibit a severe NER deficiency (47). However, a low residual activity is always present in the latter two. In fact, the XP-D complementation group in particular is characterized by heterogeneity of the repair defect, with a level of residual repair synthesis of >50% in some cases. Single point mutations are found in the *XPD* gene of XP, XP/CS and TTD patients. An inactivating deletion or truncating mutation in *XPD* is incompatible with the essential transcription function of the protein. Similarly, the moderate UV sensitivity and intermediate repair synthesis typical of XP-F patients could be due to the anticipated dual function of the XPF/ERCC1 complex in NER and repair of interstrand crosslinks

(52). A null allele for XPF or ERCC1 and the consequential defect in crosslink repair may be incompatible with life. Finally, the XPC protein is required only for GG-NER. XP-C patients display susceptibility to sunburn in the wild-type range because the causative transcription-blocking lesions (53) are removed normally (54). XP-C cells have a residual repair synthesis of 15–30% derived from TC-NER, and are less sensitive to UV than XP-A or XP-D cells (47).

Cockayne syndrome

CS is characterized by cutaneous photosensitivity, and CS cells display increased sensitivity to a number of DNA-damaging agents including UV, due to a defect in TC-NER. Surprisingly, CS patients are apparently not predisposed to develop skin cancer. Furthermore, CS is a very pleiotropic disorder with physical and mental retardation (47,55). In general, CS patients display skeletal abnormalities such as bird-like face, dental caries and kyphosis of the spinal cord, and osteoporosis in older patients. Furthermore, severe early onset progressive neurological degeneration is observed with delayed psychomotor development, gait defects and mental retardation. Microcephaly is noted in most CS patients over 2 years of age, and nerve biopsies showed myelination abnormalities. Other typical CS symptoms include sensorineural hearing loss, pigmentary retinopathy, wizened facial appearance, thin hairs and cataracts. CS individuals display impaired sexual development and postnatal growth failure and, because weight is affected more than length, the condition is termed cachectic dwarfism. The mean age of death is 12.5 years and the main causes of death are pneumonia and respiratory infections, which could well be due to the generally poor condition of the patients (55).

Many of the clinical symptoms of CS are difficult to explain via a partial NER defect, considering the fact that completely NER-deficient XP-A patients do not exhibit them. The transcriptional engagement of CSA and CSB suggests that transcription deficiency, perhaps induced by DNA damage, also contributes to the clinical symptoms. In addition, endogenous (oxidative) DNA damage has been implicated in the onset of developmental defects in CS. CSB knockout mice display only mild CS symptoms, but completely repair-deficient CSB/XPA double mutant mice suffer from severe growth failure and die before weaning (56; G.T.J.van der Horst, unpublished data). A defect in transcription-coupled repair of oxidative DNA lesions was detected in CS cells, but not in XP-A cells (57). Consequently, CS cells are slightly more sensitive to oxidative-damage-inducing ionizing radiation than wild-type cells (57). XP complementation group G contains some patients with the combined features of XP and CS. Defective transcription-coupled repair of oxidative base damage was found to be specifically confined to cells from these patients but not from XP-type XP-G patients (46), strengthening the link between defective TC-repair of oxidative lesions and the CS features.

Trichothiodystrophy

Sulfur-deficient brittle hair and ichthyosis (scaling of the skin) in combination with mental and physical retardation is referred to as trichothiodystrophy (TTD), emphasizing sulfur-deficiency of the hairs as the hallmark of the heterogeneous clinical entity to include TTD patients without ichthyosis (58). Owing to its relative rareness and broad clinical heterogeneity, many syndromes have been described that retrospectively belong to the spectrum of TTD. These include Pollitt syndrome, Tay's syndrome, Amish brittle hair syndrome, Sabinas syn-

functional allele and a disease-specific causative allele, mostly leading to single amino acid substitutions (72) (see Figure 3 for an overview). No clear correlation of disease phenotype and mutations in conserved protein domains is apparent. XP-D patients are classical XP patients with neurologic and pigmentation abnormalities, and are predisposed to develop skin cancer. XP-type XP-D fibroblasts are among the most sensitive of the different XP complementation groups, comparable with XP-A cells. Nevertheless, most XP-type XP-D fibroblasts have residual repair activity of 15–30%. Only two cases of XP-D/CS have been described with severe CS symptoms but also typical XP pigmentation abnormalities (73). Skin cancer development was observed in one patient, and relatively high unscheduled DNA synthesis (UDS) (30–40%) in the XP/CS cells is associated with high UV sensitivity (74,75). The UDS of most photosensitive TTD fibroblast lines is in the same range as XP-type XP-D (15–25%), but in general, TTD fibroblasts are less sensitive to UV-induced cell killing than XP-D cells. A subclass of TTD fibroblasts exists with relatively high UDS (40–55%) and near wild-type UV-survival. No correlation between the severeness of the repair defect and of the clinical symptoms has been found. Recently, it was demonstrated that photosensitive TTD cells have defective CPD repair but (partially) proficient repair of 6-4PPs (76) and that CPDs are the predominant mutagenic lesions in TTD cells (77). Lesion-dependent efficiency of repair may thus underlie the mild sensitivity of NER-deficient TTD cells. Other repair-related parameters that differ between XP and TTD are the UV-induced mutation spectrum (78,79), UV-induced reduction of ICAM-1 expression (80), and cellular catalase activity, which is reduced in XP but not TTD cells (81). Taken together, the TTD repair defect differs from the XP-type XP-D repair defect, which may underlie absence of cancer development in TTD patients. Other factors that may influence cancer predisposition in TTD are the reduced life span, frequent hospitalization, and hyperkeratotic skin of TTD patients, which may protect against UV. To explain the remarkable clinical heterogeneity associated with mutations in the *XPD* gene, the transcription/repair syndrome hypothesis was put forward (82,83). Mutations in *XPD* may affect the NER function of TFIIH, resulting in photosensitivity and in cancer development in XP-type patients, but may also affect the basal transcription function of TFIIH, accounting for the typical TTD and CS phenotypes. Non-UV sensitive TTD can be explained by the repair/transcription syndrome as mutations in TFIIH or other transcription factors, which only affect the transcription function. Consistent with this idea all causative mutations in *XPD* have been found to be disease-specific (Figure 3). Finally, a recently generated mouse model for TTD, mimicking a single *XPD* point mutation of a TTD patient in the mouse germ line strongly supports the hypothesis that part of the TTD symptoms are due to a transcriptional problem (84). Interestingly, exposure of these mice to UV or the chemical carcinogen 7,12-dimethylbenz[*a*]anthracene revealed cancer predisposition (albeit less pronounced as found with XPA mice) confirming the idea that a defect in DNA repair in general predisposes to cancer (85).

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