REVIEW

The xeroderma pigmentosum group D (*XPD*) gene: one gene, two functions, three diseases

Alan R. Lehmann[1]

MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR, UK

DNA, the genetic material in all living organisms, is continually exposed to agents that cause damage to its structure, resulting in the loss of vital genetic information. To counteract the potentially devastating effects of such damage, all organisms have evolved a series of different repair processes, with which many kinds of damage to the DNA can be corrected. The importance of DNA repair is shown by the existence of several human genetic disorders that are caused by defects in one of these repair processes. Thus, for example, most individuals with xeroderma pigmentosum (XP) are unable to repair damage generated in DNA by ultraviolet (UV) light from the sun, whereas patients with hereditary nonpolyposis colon carcinoma are defective in the repair of mismatched bases.

XP was the first DNA-repair disorder to be identified. It is a rare autosomal recessive genetic disorder characterized by numerous skin abnormalities ranging from excessive freckling to multiple skin cancers (Fig. 1a) (Bootsma et al. 1998). The incidence of skin cancer is about 2000-fold greater than in normal individuals. All skin abnormalities result from exposure to sunlight and are caused by inability to repair DNA damage induced in the skin by sunlight. The more severely affected patients have neurological abnormalities caused by premature neuronal death. Cells from XP donors are hypersensitive to killing by UV irradiation, and this is caused, in the majority of cases, by defects in nucleotide excision repair (NER), the process with which UV-induced photoproducts in the DNA are removed and replaced (Friedberg et al. 1995). XP is genetically heterogeneous. There are eight complementation groups designated XP-A through G and XP-variant. The *XPD* gene, defective in XP individuals assigned to the XP-D complementation group, is the topic of this review.

XPD phenotypes

Xeroderma pigmentosum

Studies performed in several laboratories have shown that cell lines from patients in each XP complementation group in general have features characteristic of the group, although exceptions have been reported (Hoeijmakers 1993). XP-D patients have severe clinical features, but less so than those of XP-A individuals, who (1) have the most marked clinical abnormalities with very severe skin changes; (2) have neurological abnormalities that manifest at an early age, typically at seven years; and (3) are totally deficient in NER. In XP-D patients, the incidence of skin cancers is markedly elevated over that of the general population, but less than that in XP-A patients. XP-Ds also have neurological abnormalities (Johnson and Squires 1992), but the age of onset is typically in the teens or early twenties. Cellular sensitivity to killing by UV irradiation is less marked than that of XP-A cells, but more pronounced than that of cells from most of the other complementation groups.

NER can be measured in several different ways, but is often assessed using the technique of unscheduled DNA synthesis (UDS), in which repair synthesis is measured quantitatively by the incorporation of ^{3}H thymidine into cells that are outside the S phase of the cell cycle and is hence unscheduled. UDS takes place in all phases of the cycle. A surprising finding is that XP-D cells have relatively high levels of UDS when compared with other complementation groups (Johnson and Squires 1992). Despite their marked hypersensitivity to killing by UV, UDS levels are typically 20% to 45% of those in normal cells, whereas cells from other complementation groups that are less sensitive to killing by UV have lower levels of UDS. This anomaly has been known for many years, but there is still no satisfactory explanation for it.

XP combined with Cockayne Syndrome

Cockayne Syndrome (CS) is another genetic disorder associated with sun sensitivity. It is a multisystem disorder characterized by severe cachectic dwarfism, mental retardation, microcephaly, and retinal and skeletal abnormalities (Fig. 1c) (Nance and Berry 1992). Whereas the neurological abnormalities that occur in some XP patients result from primary neuronal degeneration, the progressive neurological dysfunction in CS is caused by demyelination. CS patients do not have the skin pigmen-

[1]**Corresponding author.**
E-MAIL a.r.lehmann@sussex.ac.uk; FAX 44-(0)1273-678121.
Article and publication are at www.genesdev.org/cgi/doi/10.1101/gad.859501.

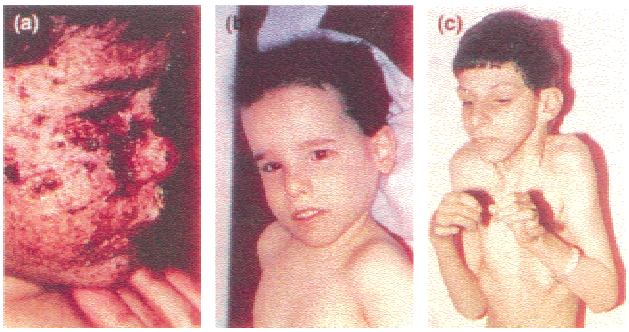

Figure 1. Patients with XP (*a*), TTD (*b*) and CS (*c*). Note the lack of skin pigmentation changes in CS and TTD compared with XP. Generously provided by A. Serasin (*a,b*) and D. Atherton (*c*).

tation changes seen in XP patients, and there are no reports of skin cancer associated with CS. Like XP cells, CS cells are sensitive to killing by UV, but unlike XP cells, they are not deficient in removing photoproducts from the bulk of the DNA. They have a specific defect in the preferential repair of damage from actively transcribed regions of DNA, a process termed transcription-coupled repair (Van Hoffen et al. 1993). Although most CS patients do not have any of the clinical features of XP, there are rare cases of individuals with the combined symptoms of XP and CS (XP/CS). Two such patients have been reported in the XP-D group (Lafforet and Dupuy 1978; Broughton et al. 1995).

Trichothiodystrophy

The principal hallmark of trichothiodystrophy (TTD) is sulphur-deficient brittle hair, caused by a greatly reduced content of cysteine-rich matrix proteins in the hair-shafts. This is accompanied by mental retardation, unusual facies, ichthyotic skin, and reduced stature (Fig. 1b) (Itin and Pittelkow 1990). Many, but not all patients, with TTD are sensitive to sunlight, but they do not have any unusual pigmentation changes, and there are no reports of cancer in TTD patients. The features of TTD have little in common with those of XP. The finding that cells from a group of sun-sensitive TTD donors were sensitive to killing by UV light and defective in NER, just like XP cells, was therefore most unexpected (Stefanini et al. 1986). Even more surprising were the results of genetic analysis, which showed that all but two of the repair-defective TTD cell lines that have been examined can be assigned to the XP-D group (Stefanini et al. 1986, 1993). An extensive analysis showed that the repair defect in different TTD individuals was heterogeneous, ranging from very severe (<10% of normal repair level) through mild (50% of normal) to no defect at all in those individuals that were not sun-sensitive (Broughton et al. 1990; Stefanini et al. 1992). These findings pose a paradox, namely, that defects in a single gene, *XPD*, result in similar cellular defects in DNA repair, but in three very different clinical phenotypes, XP, XP/CS, and TTD.

The *XPD* gene was cloned by Weber et al. (1990), and all the defective phenotypes of *XPD* cells could be corrected by introduction of the *XPD* cDNA into the defective cells (Marionnet et al. 1996). *XPD* is the ortholog of the *RAD3* gene of *Saccharomyces cerevisiae*, and the *rad15* gene of *Schizosaccharomyces pombe*. The sequence identity between these orthologs is very high (>50% amino acid identity). All three orthologs have the seven domains characteristic of DNA helicases, and both Rad3p (Sung et al. 1987) and XPD have ATP-dependent DNA helicase activity (Sung et al. 1993). *RAD3* was also known to have an essential function (Higgins et al. 1983; Naumovski and Friedberg 1983). The nature of this essential function and the clue to explaining the paradox of three disease phenotypes resulting from mutations in a single gene were revealed by the discovery of unanticipated links between DNA repair and transcription.

TFIIH, a link between transcription and DNA repair

Transcription of DNA into mRNA in eukaryotic organisms is performed by RNA polymerase II. Initiation of transcription at promoter sites requires several basal transcription factors. One of these, designated TFIIH, is composed of nine subunits. This protein complex is present at very low levels in the cell, but when the largest (89 kD) subunit was eventually isolated and sequenced, it turned out to be identical to the product of the *XPB* gene cloned previously (Schaeffer et al. 1993). Furthermore the second largest (80 kD) subunit was identical to the XPD protein (Schaeffer et al. 1994). Micro-injection of TFIIH into XP-B or XP-D cells was able to correct the repair defect in these cells (van Vuuren et al. 1994). Similarly, addition of TFIIH to cell-free extracts from XP cells restored repair activity to these extracts (Drapkin et al. 1994; Schaeffer et al. 1994). The conclusion from these findings was that the TFIIH complex has two quite independent functions, in initiation of basal transcription and in DNA repair.

These results provided a provocative hypothesis to explain how different defects in the *XPD* gene could give rise to the different clinical entities. Bootsma and Hoeijmakers (1993) proposed that if the defect affected the DNA repair function of TFIIH without affecting its transcriptional role, the features of XP would result. On the other hand, if the transcriptional role of TFIIH was affected, the consequence would be the developmental defects found in TTD (Bootsma and Hoeijmakers 1993).

The role of XPD within the TFIIH complex

To understand fully how alterations in XPD exert their many effects and how alterations in the structure of XPD affect its functions differentially, the detailed molecular architecture of the TFIIH complex is being determined. Interpretation of the results of this analysis has proved to be rather complex.

The roles of TFIIH in transcription and in excision-repair appear, at first sight, to be quite similar. In tran-

scription, it is required for opening up of the DNA at the promoter site, thus facilitating access of the RNA polymerase II transcriptional machinery (Holstege et al. 1996; Tirode et al. 1999), synthesis of the first phosphodiester bond (Holstege et al. 1997), and escape of the polymerase complex from the promoter site. In NER, its function is to open up the DNA around the damaged site to permit damage-specific nucleases to cleave the DNA on either side of the damage (Evans et al. 1997), so that the damaged section can be removed. The roles of XPD protein in these two processes are, however, quite different. It was already known that a mutation that destroyed the ATP-binding site (and by implication the helicase activity) of the yeast Rad3 protein had no effect on viability of the yeast, even though it abolished NER (Sung et al. 1988). TFIIH from this mutant was active in transcription (Feaver et al. 1993). Likewise, TFIIH complexes from cells containing XPD mutated at its ATP-binding site (lys 48→arg) had no XPD helicase activity. In vitro transcription and promoter opening were unaffected by this active site mutation, whereas cleavage on either side of damaged DNA was abolished in an in vitro DNA repair assay. Thus DNA repair is dependent on the helicase activity of XPD, whereas transcription is not (Winkler et al. 2000)

The helicase activity of XPD unwinds DNA in the 5′–3′ direction (Schaeffer et al. 1994) but its activity in the isolated XPD protein is fairly low. The XPD protein interacts physically with the N-terminal 236 amino acids of the 44-kD subunit of the TFIIH complex, designated p44 (Coin et al. 1998). This interaction results in a 10-fold stimulation of the helicase activity of XPD. Several patient mutations that have been identified in the C terminus of the gene (see below) hardly affected helicase activity of the isolated XPD protein. However, the interaction of XPD with p44 and the stimulation of helicase activity were abolished by these mutations. Thus, the helicase activity of the mutant proteins within the TFIIH complex was much lower than in TFIIH from normal donors, and this reduced helicase activity is likely to be the cause of the repair deficiency in the cell lines containing these mutations (Coin et al. 1998). Although mutations in the N-terminal region of p44 abolished this interaction with XPD, they did not affect its ATPase activity. It thus appears that one of the functions of p44 is to harness ATP hydrolysis by XPD to its helicase activity (Seroz et al. 2000)

TFIIH is made up of a total of nine subunits. Five of these (XPB, p62, p52, p44, and p34) form a tight core subcomplex. XPD is less tightly associated with the core and mediates the binding of the CAK subcomplex, which contains the remaining three subunits, cyclin H, cdk7, and MAT1 (Drapkin et al. 1996; Reardon et al. 1996). Structural studies, using electron microscopy, revealed that the TFIIH core together with XPD formed a ring-like structure with a central cavity, which could accommodate double-stranded DNA. XPD and XPB flanked the p44 subunit, and the CAK proteins formed a protuberance from the ring in the region of XPD/p44/XPB (Chang and Kornberg 2000; Schultz et al. 2000). Investigation of the roles of the different subcomplexes in an in vitro transcription system showed that the core complex was absolutely required to initiate transcription (Reardon et al. 1996). In contrast, in this in vitro system, XPD was dispensable, but in its presence transcriptional activity was substantially stimulated (Reardon et al. 1996).

The conclusion from these studies is that within the TFIIH complex, XPD needs to be present to maintain the stability of the complex for transcription initiation and promoter escape to take place, but the helicase does not need to be active.

Complex genotype-phenotype relationships in the XPD gene

Genotype–phenotype relationships in the *XPD* gene have turned out to be exceedingly complicated. The work described in the previous section has shown that for the essential role of TFIIH in transcription, the main function of the XPD protein is to maintain the stability of the TFIIH complex. As a consequence, the *XPD* gene is rather tolerant of mutations, and indeed many different viable mutations have been detected in patients in the XP-D group (Broughton et al. 1994; Takayama et al. 1995, 1996; Kobayashi et al. 1997; Taylor et al. 1997; Botta et al. 1998). For its DNA repair function, however, XPD needs to be not only present but also enzymatically active. This explains why the mutations found in XP and TTD patients result in major repair deficiencies while having only minor, if any, effects on transcription, thus permitting the cells to remain viable. Analogous experiments with XPB show that, in contrast to XPD, it is an essential part of the core TFIIH complex. XPB is also a DNA helicase, but the XPB helicase activity is essential for both transcription and repair functions (for example, see Coin et al. 1999). Thus, XPB can only tolerate very few mutations that are compatible with life.

The hypothesis that XP is a repair syndrome and TTD a transcription syndrome requires the mutations associated with the two disorders to be located at different sites in the gene. Identical mutations at a few loci were, however, found in both XP and TTD patients. This appeared to be incompatible with the idea that each mutational site is disease-specific. However, interpretation of the data is complicated by the fact that many of the patients are compound heterozygotes. A further complication is that *XPD*, because of its transcriptional role is an essential gene, and some of the alleles in compound heterozygotes were found to be lethal, probably representing total inactivation of the protein encoded by these alleles. The mutations at loci found in both XP and TTD patients are all of this lethal class and probably do not contribute to the phenotype, which is determined by the other, less severely affected, allele (Taylor et al. 1997). When this is taken into account, each mutated site is indeed found in either XP or TTD or XP/CS individuals (Fig. 2). There is no example in which identical changes were found in both alleles in XP and TTD patients. Most

of the mutations in TTD donors are localized to four sites (arg 112→his, arg 658→his or cys, arg 722→trp, –1 frameshift at codon 730). In contrast, ~75% of mutations in XP patients are localized to one site, arg 683 (Taylor et al. 1997). The mutation spectrum is therefore consistent with the hypothesis that the site of the mutation in the *XPD* gene determines the clinical phenotype. It is evident from Figure 2, however, that there are no disease-specific domains. Most (but not all) of the mutations for all three disorders are clustered in the C-terminal third of the protein, with different phenotypes in some cases resulting from changes of adjacent amino acids. For example, two XPs, one with very mild features, are mutated at arg 601, whereas alteration of the adjacent gly 602 to arg results in the very severe XP/CS phenotype. The C-terminal third of the protein, as described above, is involved in the interaction with the p44 subunit, which stimulates the activity of the helicase (Coin et al. 1999). This provides a ready explanation for the repair deficiency in cells with mutations located in this area, but it does not provide an explanation for the different phenotypes of closely located mutations. Hopefully, when the three-dimensional structure of the TFIIH complex is solved at high resolution, the precise interactions of each amino acid in this region will assist in our understanding of the effects of individual mutations.

Curiously, there are no reports of mutations between amino acids 260 and 460, even though this region is highly conserved with 50% amino acid identity between human XPD and *S. cerevisiae* Rad3p. One possible explanation for this is that mutations in this region do not affect the repair function of the protein and therefore would not result in clinical features that would be recognized as a disease entity. However, mutations generated at three conserved arginine residues in this region failed to rescue the repair activity of defective cells, so they evidently do affect repair function of the protein (M. Berneburg and A.R. Lehmann, unpubl.). The explanation for the lack of mutations in this region remains unclear.

Further complexities in genotype-phenotype relationships were revealed in an analysis of Italian TTD patients, many of whom contained the mutation arg 112→his (see Fig. 2). Some of them had only moderate clinical features, whereas in others the symptoms were very severe, even though there was a similar very marked repair defect in all of them. The analysis showed that the mildly affected group were homozygous for the arg 112→his mutation (i.e., they had two copies of the mutated gene), whereas members of the more severely affected group were functionally hemizygous with only one copy of the arg 112→his mutation, the other copy of

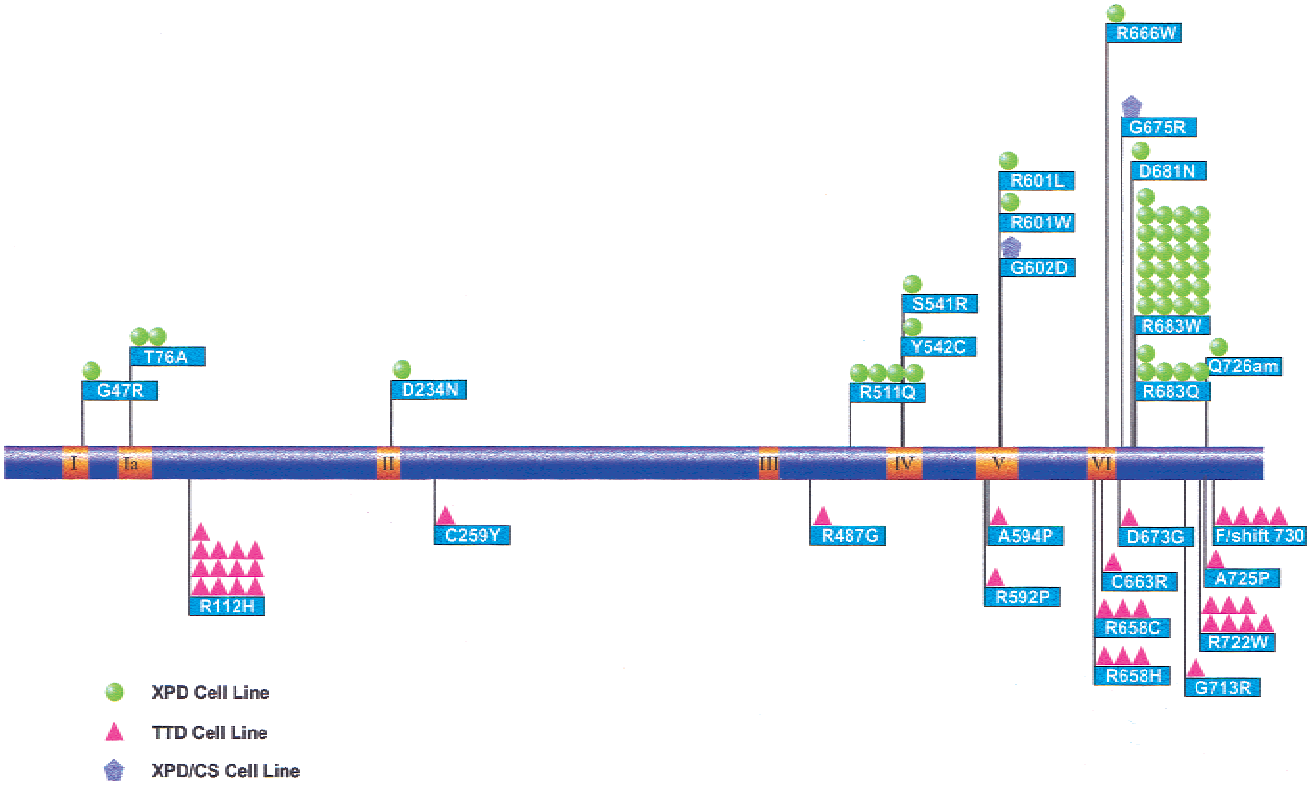

Figure 2. Distribution of mutations in XP-D patients with different disorders. The XPD protein is shown as a bar with the seven helicase domains indicated. The flags denote the amino acid changes in different patients, with the number of symbols indicating the number of times that the particular allele has been identified in patients (i.e., two symbols for homozygotes, one for compound heterozygotes). (Green circles) XP; (magenta triangles) TTD; (purple pentagons) XP/CS. Alterations considered to be completely inactivating (Taylor et al. 1997) are not shown on this figure.

the gene being completely inactive. Thus, not only the site of the mutation, but also the gene dosage, appears to affect the clinical outcome in TTD individuals (Botta et al. 1998). The XPD protein is not normally rate-limiting for transcription, but it is possible that when its activity is reduced by a mutation, which, for example, reduces its stability within the TFIIH complex, it may become rate-limiting.

Reduced levels of TFIIH in XP-D cell lines were first reported by Satoh and Hanawalt (1997). A more extensive survey has revealed that the level of TFIIH is substantially reduced in fibroblasts from all TTD individuals examined and, to a lesser extent, in some but not all XP fibroblasts (in the XP-D group) (E. Botta, A.R. Lehmann, and M. Stefanini, unpubl.). This suggests that reduced TFIIH content as a result of the instability of TFIIH is a necessary but probably not a sufficient condition for TTD to result.

The effects of mutations on the stability of XPD in the TFIIH complex may help to explain not only the three different clinical phenotypes but also the variation in severity within one clinical entity. The exact way in which each individual mutation affects the interaction of XPD with other subunits of the TFIIH complex may determine its precise effect on transcription capability and, therefore, the severity of the clinical features. A fascinating example of this has been found recently (W. Vermeulen and J.H.J. Hoeijmakers, pers. comm.). Two unrelated TTD patients had repeated episodes of pneumonia, during which they lost all their hair. The hair subsequently grew back when the fever had subsided. Both patients had the identical mutation, resulting in arg 658→cys. Cells from one of these patients were examined in detail and found to have very low levels of XPD protein. More strikingly, when the cells were cultured at 41°C, both global transcription and NER were dramatically reduced, whereas they were barely affected in normal cells. These results suggest that arg 658→cys specifically results in a temperature-sensitive phenotype.

The TTD mouse

Because TFIIH is essential for basal transcription, a vital function in every cell, it was expected that *XPD* would be an essential gene and that mice in which this gene was deleted would not be viable. This was shown to be the case (de Boer et al. 1998b). To determine if the site of the mutation really determined the phenotype, de Boer et al. selected the mutation arg 722→trp, which has been identified in five TTD families (see Fig. 2), to generate a mutant mouse. The remarkable outcome was that the mice had many of the features of TTD individuals (Fig. 3; de Boer et al. 1998a). The hair was brittle and fell out and then underwent successive growth and loss cycles. As in human TTD patients, there was a deficiency in sulphur-rich matrix proteins in the hair of the mice. Cultured cells from the mice had intermediate levels of UV sensitivity and defects in DNA repair similar to human cells carrying the identical mutation. The features of this mouse proved unambiguously that the site of the mutation determined the phenotype and, furthermore, showed that this single mutation was sufficient to generate the TTD phenotype. Furthermore, as predicted, the mouse had subtle defects in transcription in some of its tissues (de Boer et al. 1998a).

Figure 3. TTD mouse. Note the hair loss and small size of the TTD mouse, when compared with the normal mouse. Generously provided by JHJ Hoeijmakers.

It might be anticipated that a deficiency in basal transcription resulting from a subtle change in TFIIH would result in rather general abnormalities in patients or mutant mice. In fact, however, the deficiencies, for example, in cysteine-rich matrix proteins of the hairshafts, are very specific. De Boer et al. (1998a) suggested that TTD-specific mutations like arg 722→trp destabilize the TFIIH complex (see above) and that the resulting subtle defect in transcription is most marked in terminally differentiated tissues in which the mutated TFIIH might get exhausted before the transcriptional program has been completed. Hair is one such tissue. Further support for this hypothesis comes from our recent findings that almost all TTD individuals with mutations in the *XPD* gene have β-thalassemia trait, with elevated levels of haemoglobin A2 and reduced levels of synthesis of β-globin (V. Viprakasit, D.R. Higgs, and A.R. Lehmann, unpubl.). These abnormal patterns of globin synthesis are most likely a consequence of the transcriptional deficiency, and erythrocytes are, of course, another example of a tissue at the end of a terminal differentiation pathway.

Further analysis of the TTD mouse has uncovered a new role for the *XPD* gene, namely, in the ageing process. Apart from the recognized features of TTD described above, the mice have a range of symptoms associated with premature ageing: reduced lifespan, cachexia, osteoporosis, osteosclerosis, kyphosis, sexual developmental abnormalities, sebaceous gland hyperplasia, and other features of cutaneous aging such as early graying (de Boer and Hoeijmakers 1999). The way in which XPD is responsible for prevention of the aging process remains to be determined.

The developmental defects in TTD can be explained by the defect in transcription. The lack of pigmentation changes in the skin and the lack of skin cancer in TTD patients, in stark contrast to individuals with XP despite the similar defects in DNA repair, are, however, less readily explained. Several groups suggested that the re-

pair defect in TTD cells was less severe than in XP cells (for example, Eveno et al. 1995), but at least for those TTD cells containing the arg 112→his mutation, the repair defect was indistinguishable from that of XP-D cells. It is therefore likely that, in TTD, it is the transcriptional abnormality that somehow prevents the full development of a precarcinogenic lesion into a frank cancer (Berneburg et al. 2000a). Unlike the TTD patients, the TTD mice were more susceptible than normal mice to UVB-induced skin carcinogenesis (de Boer et al. 1999). This is not inconsistent with the above proposal. If the hypothesis that the transcriptional deficiency inhibits carcinogenic development is correct, this is likely to be a rather subtle interplay of complex processes, and the outcome of this balance might well vary between different species. A further possibility to explain the proneness of the TTD mouse to cancer is the reduced repair of UV-induced cyclobutane pyrimidine dimers in rodent cells. Although both human and rodent cells can remove these photoproducts efficiently from transcribed genes by transcription-coupled repair, they are excised from the bulk of the genome in human cells, but not in rodent cells. Because unexcised pyrimidine dimers are mutagenic, this may contribute to the apparently greater proneness of TTD mice to cancer as compared with that of TTD individuals (see Tang et al. 2000).

We have recently analyzed the cellular and molecular deficiencies in a patient with mild XP features, a mild repair defect, and some clinical features characteristic of TTD—the hair has a slightly reduced sulphur content, but it is not brittle (B.C. Broughton, R. Davidson, K.H. Kraemer, and A.R. Lehmann, unpubl.). This may represent a case in which a very slight transcriptional defect results in some development abnormalities typical of TTD but still permits the development of some of the skin abnormalities characteristic of XP. To date, this patient, with features of both disorders, is unique. In other cases, XP and TTD features appear to be mutually exclusive.

The strange cases of XP-D/CS

Two individuals in the XP-D group have the combined features of XP/CS. Both patients were very severely affected clinically and have unique mutations (see Fig. 2; Broughton et al. 1995; Takayama et al. 1995). Cells from these patients are exquisitely sensitive to killing by UV light, even more sensitive than XP-A cells. However, most tests have shown that the magnitude of the repair defect is quite similar to that in typical XP-D cell lines, which are significantly less sensitive to cell killing (Johnson and Squires 1992; van Hoffen et al. 1999). What could be the reason for this extreme sensitivity? As part of the excision-repair process, normal cells introduce breaks into the DNA near the damaged sites, whereas XP-D cells are unable to introduce these breaks. Surprisingly, in view of their repair deficiency, breaks are introduced into the DNA of the XP-D/CS cells at almost the same level as in normal cells. However, in the XP-D/CS cells, it turned out that the breaks are not at the site of the damage and are not part of the repair process. Introduction of damaged plasmid DNA into these cells resulted in breakage of the cells' genomic DNA even though the genomic DNA itself had not incurred any damage. These results suggest that, uniquely, XP-D/CS cells degrade their DNA following UV-irradiation damage (Berneburg et al. 2000b). This accounts for the extreme UV sensitivity of these cells and may explain some of the unusual features of the patients.

Apart from their abnormal response to UV-irradiation, XP-D/CS cells are also deficient in the repair of oxidative damage. XP-D/CS cells were proficient in their ability to repair 8-oxoguanine in plasmid-borne DNA when it was on the nontranscribed strand of the plasmid, but deficient if the damage was on the transcribed strand. Similar results were also obtained with CS and XP/CS cells from other complementation groups. XP-D cells from patients with XP alone did not, however, show this defect. 8-oxoguanine is normally repaired by the process of base excision repair, which is quite different from NER, so these results pointed to a specific defect in the repair of oxidative damage in transcribing DNA in CS and XP/CS cells (Le Page et al. 2000). This defect could contribute to the CS-specific features in XP/CS patients. The reason why these specific mutations in *XPD* result in the peculiar properties of the XP-D/CS cell lines remains obscure.

Mutant phenotypes of Rad3

Further complexities and insights into the functions of XPD can be inferred from analyses of the *S. cerevisiae RAD3* ortholog. Work on specific alleles of *RAD3* has revealed roles for Rad3p in processes other than NER and transcription. Montelone and colleagues (Montelone and Liang-Chong 1993; Montelone and Malone 1994) characterized two *rad3* alleles designated *rad3–101* and *rad3–102*, which were viable and had no defects in NER. They did however have elevated spontaneous mutant frequencies and an increased frequency of mitotic recombination.

A series of studies on recombination between short repeats and on retrotransposition showed that mutation of gly 595 in Rad3p to arg (gly 593 in XPD) resulted in elevated levels of both these processes (Bailis et al. 1995; Lee et al. 2000). Because similar results were obtained with specific alleles of *RAD25*, the homolog of *XPB*, it is likely that TFIIH is involved in inhibiting these processes, and this is related to destabilization of DNA molecules with free ends (Lee et al. 2000). Gly 593 in XPD is located between arg 592 and ala 594—alterations at both of these sites have been found in TTD patients. Although the role of TFIIH in these processes is poorly understood, it is conceivable that this role might be affected by mutations found in patients and might contribute to the clinical phenotype.

XPD mutations in human cancer

It has been proposed that sequence variations in DNA repair genes might confer susceptibility to cancer in the general population, although convincing evidence for

this is still lacking. The *XPD* gene has several polymorphic sites (Broughton et al. 1996; Shen et al. 1998). A recent study examined the risk and the age of onset of basal cell carcinomas (BCC) in patients who are frequently treated with UV phototherapy (Dybdahl et al. 1999). Because this treatment is generally conducted for several months per year, it has been suggested that these patients might be at an increased risk of developing skin cancer. The study by Dybdahl et al. (1999) looked at two common polymorphisms in the *XPD* gene and claimed that one polymorphism conferred a higher risk of developing BCCs and that the age of onset of BCCs in individuals with this polymorphism was significantly lower than in other individuals. This study was limited in size and the findings must be regarded as preliminary, but it suggested that the *XPD* gene might play a causative role in the generation of skin cancers not only in patients with XP but also in the normal population. Lunn et al. (2000) used a cytogenetic assay that measures a specific type of X ray-induced chromatid aberrations. They found that individuals with the more common genotype at one of the polymorphic sites in the *XPD* gene had higher levels of these aberrations. Again, this study was limited in size but hinted at a single-nucleotide polymorphism in the *XPD* gene that might predispose to repair deficiency. A third study compared the frequency of three polymorphic sites in the *XPD* gene in melanoma patients and a control group. There was a specific association with melanoma of one of the two alleles at each of these sites (Tomescu et al. 2000). All three of these studies suggested that the lys 751 allele was associated with greater risk than the gln 751 allele.

Outstanding questions

The discovery that XPD is a subunit of TFIIH, the spectrum of mutations in different patients and the generation of the TTD mouse have given important insights into the relationships between repair, transcription, development and cancer. However, many questions remain and hypotheses need to be tested.

1. What is the explanation for the relatively high level of UDS in many XP-D cell lines? It does not appear to represent functional repair, but probably represents some kind of abortive synthesis.
2. What are the different effects on the TFIIH complex of neighboring mutations in *XPD* that result in completely different phenotypes?
3. Why are no mutations found that affect amino acids 250–450?
4. What is special about the effects on TFIIH structure and function of the rare mutations that cause the unusual cellular defects resulting in the combined XP/CS features?
5. The hypothesis that the features of TTD result from subtle transcriptional deficiencies is widely accepted and very plausible. To date, however, the only direct evidence to support it is the finding of reduced levels of mRNA of the *SPRR2* gene in the skin of the TTD mouse (de Boer et al. 1998a).
6. There has been much speculation about the reasons why TTD individuals do not have the severe skin abnormalities that are the cardinal features of XP patients, even though the repair defects appear, in some cases, to be indistinguishable. I have suggested that the most likely explanation is that the transcription abnormality somehow prevents the development of the carcinogenesis program. However, there is as yet no direct evidence for this or any other hypothesis.
7. Does the genetic background have a significant effect on the clinical phenotype? The TTD mouse shows that the arg 722→his mutation is sufficient in itself to cause the TTD features. Until XP and XP/CS mice have been generated with appropriate *XPD* mutations, we cannot be certain that other genetic factors do not affect either the nature or the severity of the phenotype.
8. What causes the neurological abnormalities in XP-D (and also XP-A) patients? If an XP-D patient is diagnosed early, rigorous protection from the sun can prevent the development of almost all the skin abnormalities. Nothing can be done to prevent the neurological degeneration, and this is becoming the major problem in patient care for XP sufferers. As yet, we have no clue about the cause of the neurological abnormalities, and the intractability of the brain as an experimental system makes this problem refractory to attempts to understand these defects. A plausible hypothesis that has been proposed by several groups is that the neurological degeneration in XP results from an inability to repair some types of oxidative damage that accumulates in the brain. Although XP-D cells appear to have normal capacity to remove 8-oxoguanine (see above; Le Page et al. 2000), XP extracts are unable to repair purine cyclodeoxynucleosides, which can be formed by exposure of DNA to free radicals (Brooks et al. 2000; Kuraoka et al. 2000).

Acknowledgments

I am grateful to Elaine Taylor, Patricia Kannouche, Maria Fousteri, and Bryn Bridges for helpful comments and to Bernard Broughton for producing Figure 2.

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Alan R. Lehmann

Genes Dev. 2001, **15:**
Access the most recent version at doi:10.1101/gad.859501