



Invited Mini-review

Trichothiodystrophy: From basic mechanisms to clinical implications

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ABSTRACT

Trichothiodystrophy (TTD) is an autosomal recessive disorder with symptoms affecting several tissues and organs. The most relevant features are hair abnormalities, physical and mental retardation, ichthyosis, signs of premature aging and cutaneous photosensitivity. The clinical spectrum of TTD varies widely from patients with only brittle, fragile hair to patients with the most severe neuroectodermal symptoms. To date, four genes have been identified as responsible for TTD: *XPD*, *XPB*, *p8/TTDA*, and *TTDN1*. Whereas the function of *TTDN1* is still unknown, the former three genes encode subunits of TFIIH, the multiprotein complex involved in basal and activated transcription and in nucleotide excision repair (NER). Ongoing investigations on TTD are elucidating not only the pathogenesis of the disease, which appears to be mainly related to transcriptional impairment, but also the modalities of NER and transcription in human cells and how TFIIH operates in these two fundamental cellular processes.

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1. Introduction

Trichothiodystrophy (TTD) is a term derived from Greek (*tricho*, hair; *thio*, sulfur; *dys*, faulty; and *trophe*, nourishment) that was

Abbreviations: aa, aminoacid; CAK, cdk-activating kinase; Cdk, cyclin-dependent kinase; CR, caloric restriction; CS, Cockayne syndrome; CTD, carboxy-terminal domain; ER α , estrogen receptor α ; GH, growth hormone; IGF-1, insulin growth factor 1; NER, nucleotide excision repair; NR, nuclear receptor; PPARs, peroxisome proliferator-activated receptors; RAR α , retinoic acid receptor α ; RNAPol, RNA polymerase; TFIIH, basal transcription factor IIH; TTD, trichothiodystrophy; UDS, UV-induced DNA repair synthesis; UV, ultraviolet; XP, xeroderma pigmentosum.

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coined by Price et al. in 1980 [1] to describe a group of autosomal recessive disorders characterized by sulfur-deficient brittle hair and other neuroectodermal symptoms that commonly include mental and growth retardation, proneness to infections, ichthyosis, nail abnormalities, decreased fertility and features of premature aging. Cutaneous photosensitivity has been reported in approximately half of the cases and is associated with an altered cellular response to ultraviolet (UV) light due to a defect in nucleotide excision repair (NER), the DNA repair pathway that removes a wide spectrum of lesions, including UV-induced damage. Three genes have been identified as responsible for the photosensitive form of TTD, namely *XPB*, *XPD* and *p8/TTDA*. The discovery that these genes encode distinct subunits of the general transcription factor IIH (TFIIH), a multi-protein complex involved in both NER

and transcription, has been crucial to rationalize the TTD clinical outcome and the puzzling variety of pathological phenotypes associated with mutations in the *XPB* and *XPD* genes.

Evidence has been provided that also the non-photosensitive form of TTD is genetically heterogeneous by showing that about 18% of the analyzed cases are mutated in the *C7orf11* gene, thereafter designated *TTDN1* (TTD non-photosensitive 1). This review addresses the recent advances in the field, focusing on the still emerging understanding of the pathogenesis of TTD and of the multiple roles of TFIIH in human cells.

2. Pathological features of TTD

All TTD patients exhibit sparse, dry and easily broken hair characterized by low sulfur and cysteine content and a typical banding, called tiger-tail pattern, when observed under polarizing microscopy. Hair anomalies, which are considered the diagnostic hallmark of the disorder, are associated with a wide spectrum of clinical symptoms that usually affect organs of ectodermal and neuroectodermal origin (reviewed in [2–4]). Common features include mental and growth retardation, nail abnormalities, ichthyosis, skin photosensitivity, microcephaly, facial dysmorphism, ocular and skeletal abnormalities, recurrent infections and impaired sexual development. In addition, osteoporosis, hearing loss, cataracts, dental caries, and other features of premature aging have been reported. Delivery is frequently preterm and children may be encased in a collodion-like membrane at birth. The disorder is characterized by a wide range in type and severity of symptoms. The most severe form is characterized by very poor mental and motor performance and speech, failure to thrive and death frequently occurring during early childhood, often due to overwhelming bacterial infections. Other patients show a pathological phenotype of moderate severity, with short stature, delayed puberty, mental development at pre-school or primary school level, axial hypotonia, reduced motor coordination and survival beyond early childhood. A few mild cases have been described with involvement of only hair, nails or skin (reviewed in [3]).

The remarkable progress made over the last twenty years on TTD has revealed two distinct forms of the disorder characterized by the presence or absence of clinical and cellular photosensitivity (OMIM #601675, OMIM #234050). The incidence of the NER-defective form of TTD has been recently established at 1.2 per million live-births, based on combined data from five DNA repair diagnostic centres in West-Europe [5].

3. TFIIH and its central role in the photosensitive form of TTD

Photosensitive TTD cases are mutated in either the *XPB*, *XPD* or *p8/TTDA* genes, which encode subunits of TFIIH, a multi-protein complex that, besides participating to NER, is involved in RNA polymerase (RNAPol) II transcription initiation and regulation, RNA pol I transcription, and cell cycle control. The versatile engagement of TFIIH in distinct cellular processes might explain why mutations in *XPB* and *XPD* result in a variety of autosomal recessive disorders. In addition to TTD, these include the cancer-prone disorder xeroderma pigmentosum (XP), Cockayne syndrome (CS), a disorder that shares many features of aging and developmental anomalies with TTD, and other rare clinical entities showing XP features in combination with CS or TTD clinical symptoms. It has been suggested that this pleiotropy may be related to mutations in different sites of the *XPB* and *XPD* genes, which differentially interfere with the stability and the conformation of the TFIIH complex, thus affecting its functional activities in slightly different ways [6].

3.1. TFIIH composition and activities

Accumulating evidence indicate that the composition of TFIIH is dynamic to adapt its engagement in distinct cellular processes. The transcriptionally active form of TFIIH (holo-TFIIH) is composed of a six-subunit core (XPB, p62, p52, p44, p34 and p8/TTDA) associated with the cdk-activating kinase (CAK) subcomplex formed by the three subunits cdk7, MAT1 and cyclin H. The core-TFIIH and CAK sub-complexes are bridged by the XPD subunit that interacts with p44 on the one side and MAT1 on the other side. The structural function of XPD in TFIIH assembly is used by cells in a dynamic way to regulate and coordinate the diverse functions of the different sub-complexes in transcription, DNA repair and cell cycle progression.

TFIIH possesses several enzymatic activities: the two ATPase and DNA helicase activities of XPB and XPD, the protein kinase activity of the cyclin-dependent kinase cdk7, a key player in transcription and cell cycle regulation, and the E3 ubiquitin ligase activity of p44, which is important for the yeast transcriptional response to DNA damage [7]. These enzymatic activities are tightly modulated by interactions with many components of the transcriptional machinery, including regulatory transcription factors, as well as by contacts within the TFIIH complex (reviewed in [2,8,9]).

In the transcription of class II genes, TFIIH participates to the opening of the promoter around the transcription initiation site through the XPB and XPD ATP-dependent helicases, and contributes to efficient promoter escape by phosphorylating the carboxy-terminal domain (CTD) of the largest subunit of RNAPol II via its cdk7 kinase. TFIIH is also involved in the phosphorylation of activators, such as Ets1, and several nuclear receptors (NRs). Recent evidence indicates that optimal transactivation of the estrogen receptor α (ER α) requires the interaction of TFIIH with XPG, a structure-specific endonuclease that cleaves 3' of the DNA lesion during NER. In ER α regulation, XPG is dedicated to the stabilization of TFIIH by preventing the dissociation of CAK and XPD from the core subcomplex [10]. This new additional role of XPG provides a reasonable explanation for those clinical features present in some XP-G patients, such as hypogonadism and loss of subcutaneous fat tissue, which cannot be explained by a repair defect (reviewed in [11]).

In the first steps of NER, TFIIH opens the double-stranded DNA around the lesion, a function that depends on the 5'–3' helicase activity of XPD and the ATPase activity of XPB [12,13]. Once TFIIH has been recruited at the damaged site, the core subcomplex associates with NER-specific factors, including XPA, which catalyzes the detachment of the CAK from the core thereby triggering the incision/excision of the damaged oligonucleotide. Following damage removal, the NER factors are released from the complex and the CAK reappears with the core TFIIH on the chromatin, together with the resumption of transcription formerly inhibited by UV irradiation [12]. A further contribution of TFIIH to genome integrity is its role in the cell cycle. The free CAK subcomplex is a pivotal positive regulator of cyclin-dependent kinases that are key regulators of cell cycle progression and control [14,15].

Besides its well-established role in transcription of protein-coding genes and repair, TFIIH is also involved in RNAPol I transcription, in a step subsequent to initiation [16]. It has been shown that in human cells TFIIH moves freely and gets engaged in RNAPol I and RNAPol II transcription for about 25 and 6 s, respectively. Furthermore, TFIIH readily switches between transcription and repair sites where it is immobilized for 4 min [17]. Spatiotemporal distribution of TFIIH has been recently analyzed in a mouse model expressing fluorescently tagged TFIIH [18]. This new tool has allowed the monitoring of the TFIIH mobility in several living tissues revealing that in highly differentiated postmitotic cells, such as neurons, hepatocytes and cardiac myocytes, TFIIH is immobilized

Table 1
Clinical and cellular features of TTD patients mutated in the p8/TTDA, XPB and XPD subunits of the repair/transcription complex TFIIH.

Mutated gene	Case no. (families)	Clinical symptoms ^a				TFIIH level ^b	NER efficiency ^c	
		Hair alterations	Skin photos.	Physical impairment	Neurological impairment		UV sensitivity	UDS (% of normal)
TTDA	4(3)	+	+	+	+	30	++	15–25
XPB	2(1)	+	+	–	–	40	+	40
XPD	33(30)	+	+	+ / ++	+ / ++	35–65	+ / ++	10–50

Abbreviations: NER: nucleotide excision repair; UDS: unscheduled DNA synthesis; photos.: photosensitivity.

^a Physical impairment: (+) moderate (survival beyond early childhood, delayed puberty, and short stature); (++) severe (death during childhood and/or failure to thrive/dystrophy). Neurological impairment: (+) moderate severity (mental development at either preschool level or primary-school level, axial hypotonia, and reduced motor coordination); (++) severe (very poor mental and motor performances and speech).

^b TFIIH level refers to the mean steady state of the subunits cdk7, p44 and p62 in patient cells expressed as percentage of that in normal cells analysed in parallel.

^c NER efficiency refers to the cellular response to UV. UV-sensitivity: survival partially (+) or drastically (++) reduced compared with normal. UDS: ability to perform UV-induced DNA repair synthesis expressed as a percentage of that in normal cells.

on the chromatin at sites of RNApol I and RNApol II transcription. This immobilization is both differentiation driven and development dependent. Although statically bound, TFIIH can be rapidly remobilized in response to a genotoxic or environmental stress that requires a rapid adaptation of the transcriptional program.

3.2. Function of the genes responsible for the photosensitive form of TTD

The three TFIIH subunits mutated in TTD have different roles. XPB and XPD are ATP-dependent helicases with opposite polarity and participate in the unwinding of DNA both in repair and basal transcription [19,20]. The 3'–5' helicase activity of XPB is essential for transcription whereas the 5'–3' helicase activity of XPD is dispensable for *in vitro* basal transcription, although XPD facilitates optimal transcription by anchoring the CAK subcomplex to the core TFIIH [21]. This probably accounts for the rarity of XP-B patients compared with the relatively high frequency of pathological phenotypes associated with mutations in XPD.

In NER, the efficient opening of the DNA around the damage is driven by the helicase activity of XPD [21], which is regulated by the TFIIH subunit p44 [22], and by the ATPase activity of XPB, which is regulated by the TFIIH subunit p52 [20] and the damage recognition factor XPC [23]. It has been shown that the archaeal XPD helicase is able to motor along on DNA coated with ssDNA-binding proteins, and it can either displace proteins it encounters or it can slip right past them without either protein falling off of the DNA [24]. This may represent a generalized mechanism employed by the XPD/Rad3 family helicases for targeting the cognate DNA-processing intermediates.

The p8/TTDA subunit has been identified only recently, by showing that the four NER-defective TTD patients representative of the TTD-A group were mutated in the human homolog of the yeast gene encoding a new small 8 kDa component of TFIIH [25,26]. Through interactions with the subunits p52 and XPD, p8/TTDA provides stability to the entire complex and it is a key regulator of the TFIIH cellular levels *in vivo* [27,28]. Furthermore, p8/TTDA is dispensable for basal transcription whereas it has a pivotal role in DNA repair [27]. Following DNA damage, p8/TTDA forms a more stable association with TFIIH in nuclei [28] and promotes the local opening of the DNA around the lesion by stimulating the ATPase activity of XPB together with p52 and the damage recognition complex XPC-hHR23B. In addition, p8/TTDA promotes the translocation of XPA to UV damaged sites [20,27].

3.3. Mutational pattern and its relation with the clinical and cellular features

The reported investigations on thirty-nine NER-defective TTD patients indicate that most of them are mutated in the XPD gene

whereas mutations in either the XPB or p8/TTDA gene account for a minority of cases (Table 1). These patients display different degrees of clinical severity, repair defect and reduction in the cellular amount of TFIIH, aspects that will be discussed in the context of each genetic defect.

The p8/TTDA gene encodes the only TFIIH subunit for which a complete absence is compatible with life, indicating its negligible role on the transcriptional activity of the complex [26]. As illustrated in Fig. 1, two of the mutations detected in TTD-A patients result in the absence of the protein or in non-functional truncated peptides. The third mutation converts the conserved leucine residue at position 21 to a proline and it is localized in one of the hydrophobic patches on the protein surface that might be involved in the recognition of the molecular partners [29,30]. The four TTD-A patients show a pathological phenotype of mild/moderate severity, a drastically reduced ability to repair UV-induced DNA damage (15–25% of normal) and a notable reduction in the TFIIH cellular amount (30% of normal) [26,31–33]. The overall features of TTD-A patients suggest that a two-third reduction in the amount of an otherwise normal TFIIH complex drastically hampers NER efficiency but confers only subtle defects in transcription resulting in clinical features of mild/moderate severity [32]. As well as implying that NER requires higher concentrations of TFIIH than transcription does, these findings underline the relevance of p8/TTDA for the TFIIH activity in DNA repair.

The two TTD siblings mutated in the XPB gene are homozygous for a mutation resulting in the p.Thr119Pro substitution [34] that only slightly interferes with basal transcription activity *in vitro* [35]. This finding confirms that the XPB protein tolerates only rare alterations compatible with its essential role in transcription. The Thr119Pro change is associated with a partial NER deficiency in NER and TFIIH cellular content (both corresponding to 40% of normal), and a mild pathological phenotype characterized by hair abnormalities, mild ichthyosis and mild cutaneous photosensitivity. Both patients were born at term with a similar presentation as a colloidion baby of favorable outcome and they did not show any physical and mental impairment at the ages of 10 and 16 years, respectively. The relatively mild symptoms observed in these siblings compared with those in other TTD patients have led the clinician to describe them clinically as a TTD “variant” [36].

The few other reported XPB mutations localize in different sites of the gene and are associated with either mild XP (2 cases from 1 family) or XP/CS (7 cases from 5 families) [37,38].

Mutation analysis in thirty-three TTD patients mutated in the XPD gene (TTD/XP-D) [39–46] indicates that eleven patients are homozygous for a mutation resulting in a single substitution of an arginine residue at position 112, 658, 592 or 722 (Fig. 1). The remaining twenty-two patients are compound heterozygotes with various combinations of mutated XPD alleles. Three cases are functional hemizygotes, indicating that the mutations present

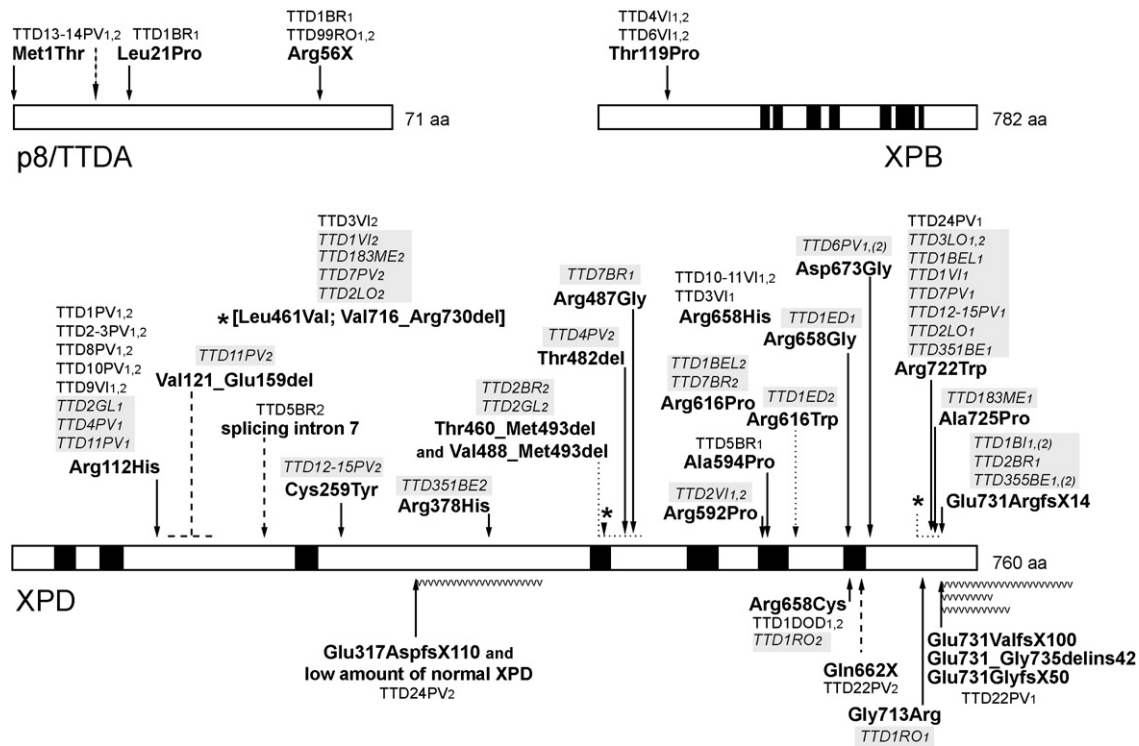


Fig. 1. Amino acid changes in the p8/TTDA, XPB and XPD proteins from mutations described in photosensitive TTD patients. The XPB and XPD proteins are shown with the helicase domains in black. The numbers 1 and 2 after the patient code denote the different alleles. In p8/TTDA, the dashed arrow indicates the downstream methionine codon at position 16 that could result in production of an N-terminally truncated polypeptide in the siblings TTD13–14PV. In XPD, the changes responsible for the pathological phenotype, those resulting in deletions likely to affect cellular viability and mutations described as lethal [42] are indicated by solid, dashed and dotted arrows, respectively. The asterisks indicate the alterations [Leu461Val; Val716_Arg730del] found associated in a single haplotype. TTD patients show a mild/moderate clinical phenotype whereas XPB patients are mildly affected. The code of TTD/XP-D patients with severe clinical features is in italic with a gray background. See Table 1 and text for details. Mutation nomenclature follows the format indicated at www.hgvs.org/mutnomen. p8/TTDA, XPB and XPD protein sequence refers to GenBank NP.997001.1, NP.000113.1, NP.000391.1, respectively.

in the only expressed allele (p.Asp673Gly in one patient and p.Glu731ArgfsX14 in two patients) are compatible with life.

Mutations are distributed across the gene and do not delineate any specific domain. Most of the changes are localized at three sites and all involve a single substitution of an arginine residue (Arg112, Arg658 and Arg722). Other frequently observed mutations are the substitution of Arg616, the Leu461Val change and the deletion of the aa region 716–730, the latter two being associated with the same allele. These two alleles are likely to be nonfunctional because they behave as null alleles in *S. pombe* [42] and they completely abolish basal transcription *in vitro* [47], a defect that is obviously incompatible with life. This explains why they have never been found in the homozygous state but always in association with other mutated *XPD* alleles that less drastically interfere with basal transcription [47]. It is worthwhile mentioning that these two nonfunctional alleles have been also detected in XP patients. The pathological phenotype in these cases appears to be determined by the mutation on the second allele that is always different in TTD and XP patients. Mutated *XPD* alleles typically associated with the XP phenotype are predicted to result in the change of the residue Arg683. These alleles are present in 26 out of the 31 reported XP cases [48,49].

The TTD/XP-D patients display different degrees of clinical severity. Some cases are moderately affected whereas others exhibit a severe clinical outcome. Also the DNA repair ability shows different degrees of alterations, ranging between 50% and 10% of normal [50–53]. The differential impairment in the cellular response to UV does not correlate with the degree of clinical severity but with the mutation site (Fig. 2). A mild NER deficiency is present in patients with mutations resulting either in the change

of Arg658 or in the loss of the final portion of the XPD protein. An intermediate NER deficiency is associated with the Arg722Trp change whereas a remarkable repair defect is associated with the Arg112His change. In TTD/XP-D patients also the TFIIH steady-state level varies from 35% to 65% of normal but again the reduction in the TFIIH amount does not correlate either with the residual repair capacity or with the clinical severity (Fig. 2).

Investigations on Italian TTD patients revealed that the Arg112His compound heterozygotes are more severely affected than the homozygotes [43], suggesting that the main determinant of the clinical severity might be the effective *XPD* gene dosage. Examination of other patients supports this hypothesis (Fig. 1). A recent investigation on TTD compound heterozygotes sharing a typical TTD allele (p.Arg722Trp) has shown that the less compromised phenotype of Patient TTD24PV was associated with a mutation in the second allele leading to the production of a low level of a normal XPD protein that is able to partially rescue the functionality of TFIIH [46]. A moderate phenotype has been unexpectedly found in a patient compound heterozygous for an allele (p.Gln662X) likely to be lethal and a lowly expressed allele with a mutation predicted to generate three distinct XPD proteins mutated from residue 731 and longer than normal [46]. This finding indicates that a change of the C-terminal 30 aa of the 760 aa XPD protein is still compatible with the function of TFIIH in repair and transcription, in agreement with the observation that this is the only part of the *XPD* gene that is poorly conserved [39]. These studies indicate that identification and characterization of patients with unusual and/or unexpected genotype–phenotype relationship represent a useful tool to gain further insights into the *in vivo* functional effects of mutated proteins and into their impact on the whole organism.

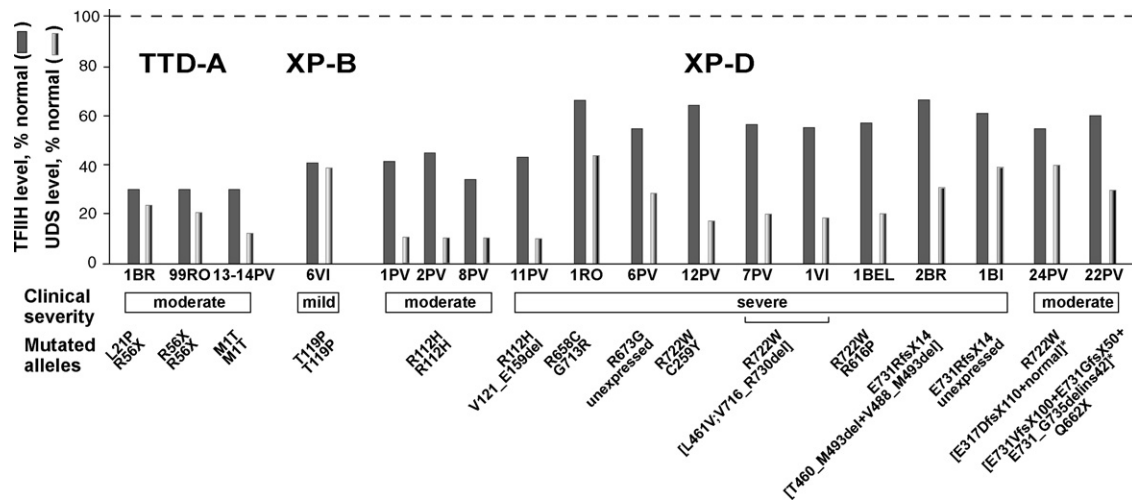


Fig. 2. Levels of TFIIH and UV-induced DNA repair synthesis, causative mutations and clinical severity in TTD patients classified into the TTD-A, XP-B or XP-D groups. The level of TFIIH was evaluated by Western Blot in cell lysates using antibodies against the subunits cdk7, p44 and p62 ([26,33,46] Botta et al., unpublished results). TFIIH values in TTD fibroblasts are the mean steady-state levels of the analyzed subunits expressed as percentages of the corresponding value in normal C3PV fibroblasts analyzed in parallel. UV-induced DNA repair synthesis (UDS) levels after irradiation with 10J/m² observed in TTD fibroblasts are expressed as percentages of the corresponding value in normal C3PV fibroblasts analyzed in parallel. All the reported values are the means of at least two independent experiments, with SE values always <10%. The asterisks indicate the lowly expressed *XPD* alleles.

A complex phenotype of moderate severity with some features of both XP and TTD (XP/TTD) has been found in four patients [45,54]. Three were compound heterozygous for a typical TTD allele (p.Arg112His or p.Arg722Trp) and for a novel allele (p.Ser51Phe, p.Leu485Pro or p.Cys663Arg) that may contribute to the XP phenotype. The remaining patient had two novel mutated *XPD* alleles (p.Phe568TyrfsX2 and p.Glu582.Lys583delins3). It has been suggested that a peculiar situation in the genetic background of this patient may mitigate the effects of transcription and NER impairment, resulting in mild TTD and XP symptoms. Alternatively, the mutation present in the less severely affected allele might confer an extremely mild defect in transcription that does not completely prevent the phenotypic consequences of the repair defect, as usually found for the mutations associated with TTD [54]. Interallelic complementation has been observed in mouse models carrying *XPD* mutations specific for distinct disorders, suggesting that null alleles may still influence disease outcome in compound heterozygotes [55].

Overall these studies indicate that the phenotypic consequences of a single mutated *XPD* allele depend on the precise balance of its effects on stability and conformation and, consequently, functional activities of TFIIH. The clinical outcome in patients will reflect the combined effects of each allele on TFIIH activity.

4. Genes responsible for the non-photosensitive form of TTD

Our knowledge on the functions altered in NER-proficient TTD patients is still very limited. Nothing is known about the functional activity of *C7orf11/TTDN1*, the gene mutated in a small proportion of non-photosensitive TTD cases that show normal response to UV light and TFIIH steady state level [56,57]. *C7orf11* is an uncharacterized open reading frame that maps to human chromosome 7p14. The corresponding protein contains a glycine/proline-rich region but no putative conserved domains, localizes to the nucleus and is expressed in the hair follicles [56]. Different levels of *TTDN1* expression were detected in different human cell-types, suggesting a regulation at the transcriptional level (our unpublished results). During mitosis *TTDN1* interacts with polo-like kinase 1 (Plk1) and is phosphorylated by cdk1 [58]. Furthermore, overexpression of *TTDN1* in HeLa cells causes nuclear fragmentation, whereas its

knock down results in multiple nuclei or multiple-polar mitotic spindles [58]. However, these alterations are not observed in cells from *TTDN1* patients (our unpublished results).

Mutations in *TTDN1* have been so far detected in three Moroccan siblings, an Amish kindred and seven unrelated patients [56,57] (Fig. 3). A missense mutation has been reported only in the Amish kindred. The gene is deleted in three patients whereas the mutations found in five cases result in frameshifts predicted to produce severely truncated proteins. This mutation pattern indicates that the *TTDN1* gene is not essential for cell proliferation and viability. Furthermore, the severity of the clinical features of the patients does not correlate with the molecular defect, indicating that other factors besides *TTDN1* mutations influence the severity of the disorder.

What can be predicted on the functions defective in the non-photosensitive TTD patients on the basis of the limited available observations? Since the two forms of TTD share all the clinical features except photosensitivity, it has been suggested that the genes responsible for the non-photosensitive form might play a

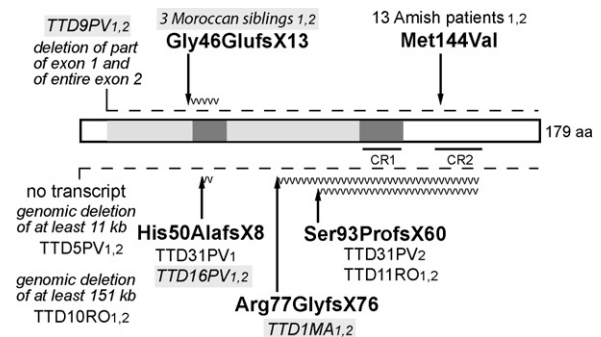


Fig. 3. Amino acid changes in the *TTDN1* protein from mutations described in non-photosensitive TTD patients. The *TTDN1* protein is shown with the glycine/proline rich region in gray (the low complexity regions detected by the BLASTP program are in dark gray) and the two highly conserved C-terminal regions (CR1 and CR2) present among the candidate orthologs. Mutations described in [56] and in [57] are shown above and below the depicted protein, respectively. The numbers 1 and 2 after the patient code indicate the different alleles. The code of patients with severe clinical features is in italic with a gray background. Mutation nomenclature follows the format indicated at www.hgvs.org/mutnomen. *TTDN1* protein sequence refers to GenBank NP.619646.1.

role in transcription regulation but not in DNA repair. The corresponding proteins could be required for optimal functioning of TFIIH in tissues/organs affected in both forms of TTD. Alternatively, they could be transcriptional regulators of genes relevant for metabolic pathways that are central to the outcome of TTD. At present, the identification of the still unknown disease-gene(s) responsible for non-photosensitive TTD cannot rely on whole-genome strategies, due to the paucity of the material worldwide available from informative patients/families. Our search has been focused on the transcriptional coactivators *PRMT1* and *CARM1* [59], two attractive candidates for the functions altered in the non-photosensitive form of TTD. However, no mutations were detected in eleven NER-proficient TTD patients who were not mutated in *TTDN1* (our unpublished results).

5. Pathogenetic basis of the TTD clinical outcome

As detailed in the previous sections, all the mutations found in NER-defective TTD patients result in reduced steady-state levels of the entire TFIIH complex, modification of its architecture and impaired functioning in repair and transcription. Progress in our knowledge of the mechanistic and functional defects underlying the TTD outcome originates from studies on XPD, which have been promoted and supported by the relatively conspicuous number of affected patients. A fascinating example demonstrating the link between TFIIH instability and TTD clinical outcome is seen in two unrelated patients who displayed reversible worsening of TTD features, such as hair loss and ichthyosis, during febrile episodes associated with intercurrent infections. Both patients had an identical mutation in the *XPD* gene (p.Arg658Cys) that was shown to confer a thermo-instability of TFIIH resulting in a temperature-sensitive defect of transcription and DNA repair [60].

Further insights into TTD pathogenesis were gained with the generation of the TTD mouse model that contains a *XPD* mutation (p.Arg722Trp) that is found in several patients with TTD but not in any with XP. The mouse had many TTD features, including brittle hair, developmental abnormalities, reduced life span, UV sensitivity and skin abnormalities [61,62], thus conclusively proving that the single alteration in the *XPD* gene was responsible for all the defects associated with TTD. Consistent with the relevant role of XPD in basal transcription, mice with a *Xpd* null allele were embryonic lethal in the pre-implantation stage [63].

Evidence supporting a transcriptional defect in TTD has been provided by *in vitro* studies with recombinant TFIIH complexes in which the XPD subunit carries aminoacid changes found in patients. All the mutations found in XP-D patients, independent of the associated pathological phenotype, affect the helicase activity of XPD, thus explaining the NER defect but only those responsible for TTD diminish the basal transcription activity of TFIIH [47]. The recent resolution of the crystal structure of archaeal XPDs revealed that the mutations associated with XP map close to the ATP-binding pocket or at sites predicted to interact with DNA, and drastically reduce the helicase activity of the XPD protein [64–66]. In contrast, the mutations found in TTD cause framework defects impacting TFIIH integrity, according with the reduced level of the TFIIH content typically present in TTD cells (reviewed in [67]).

5.1. Skin and developmental abnormalities

The transcriptional failure in TTD is compatible with life and therefore it has to occur only under certain circumstances and/or in specific cellular compartments. It is believed that quantitative and conformational alterations of TFIIH in TTD may become limiting in terminally differentiated tissues, in which the mutated TFIIH might be insufficient to provide adequate transcriptional activity

of a diverse set of highly expressed genes. In line with this suggestion, the hair abnormalities, which are the clinical hallmark of TTD, result from a deficiency in cysteine-rich matrix proteins in the hairshafts of TTD patients. Alterations in the transcriptional program in late stages of differentiation might account also for the β -globin deficiency in erythrocytes of TTD/XP-D patients showing the haematological features of β -thalassaemia trait without mutations in hemoglobin genes [44], for the alterations in T cells and dendritic cells reported in few TTD patients [50,68], and for other typical TTD abnormalities, such as ichthyosis. Indeed, a lowered expression of differentiation markers has been described in the skin [61] and *in vitro* differentiating keratinocytes [69] from the TTD mouse model.

Other clinical symptoms may be related to interference with the TFIIH regulatory role in gene expression. Some mutations in the C-terminal end of XPD have been shown to prevent the phosphorylation of specific ligand-activated NRs and to interfere with the anchoring of CAK to the core TFIIH. Namely, the mutations Arg683Trp (XP) and Arg722Trp (TTD) impair the transactivation of the retinoic acid receptor α (RAR α) and the peroxisome proliferator-activated receptors (PPARs) [47,70,71]. Nevertheless, almost optimal transactivation of RAR α was found in TTD-A cells [70] and in TTD cells with the Arg112His substitution [47] that showed also a normal transactivation of PPARs [71]. These findings offer a clue for understanding some developmental and neurological defects encountered in some XP and TTD patients. The hypoplasia of adipose tissues in TTD (p.Arg722Trp) mice might be due to a defective transactivation of PPARs, which are essential for lipid metabolism and differentiation/survival of adipocytes *in vivo* [71]. Recent studies on TTD mice have also demonstrated a selective dysregulation of thyroid hormone target genes in specific areas of the brain that results from the inability of mutated TFIIH complexes to fully participate to the recruitment/stabilization of thyroid hormone receptors on their target genes [72]. The tissue-specificity of this defect is conferred by the cell type-specific transcription regulatory machinery and not by TFIIH itself. As well as revealing an unexpected coactivator function of TFIIH, these results provide an explanation for some of the neurological manifestation of TTD since the thyroid hormone contributes to the regulation of several processes taking place during perinatal brain development, such as myelinogenesis and neuronal cell migration.

5.2. Aging features

TTD is regarded as a segmental progeroid syndrome because several patients exhibit signs of premature aging. A role for the *XPD* gene in the aging process is supported by some of the features present in the TTD mouse model (reviewed in [73]). Apart from the skin and developmental abnormalities typical of TTD, the mice show reduced lifespan, cachexia, osteoporosis, osteosclerosis, kyphosis, sebaceous gland hyperplasia, and other features of cutaneous aging such as early graying [62,74,75]. Furthermore, they exhibit progressive loss of bone marrow progenitor cells and age-dependent decline of hematopoietic stem cell function [76]. Unexpectedly, these premature aging features are accompanied by symptoms that are normally observed after dietary caloric restriction (CR) [77], the only well documented intervention known to extend life span and delay the onset of many aspects of aging in mammals. This paradox finds an elegant solution in the observation that CR as well as normal aging are characterized by a systemic attenuation of the growth hormone (GH) and insulin growth factor 1 (IGF-1) somatotrophic axis. The somatotrophic attenuation is emerging as a feature shared also by NER progeroid mouse models [78]. These observations led to the fascinating hypothesis that accumulation of DNA damage interferes with normal DNA metabolism and triggers suppression of the GH/IGF-1 somatotrophic axis and

dampening of oxidative metabolism. This is a “survival response” that shifts the energy equilibrium from growth and proliferation to pathways preserving somatic maintenance in the attempt to extend lifespan. NER progeroid mice accumulate DNA damage much more rapidly than naturally ageing mice as a consequence of their repair defect, and onset of the life extending “survival response” is accelerated [79,80].

Interestingly, in the liver of TTD mice a reduced IGF-1 signaling and a decreased energy metabolism were found associated with increased homeostatic imbalance between apoptosis and cell proliferation [81]. A failure of the organism to maintain homeostasis might explain the general conditional decline (including cachexia) that is an important cause of death in TTD mice as well as in several TTD patients.

However, the way in which XPD is responsible for prevention of the aging process remains to be determined. Several evidence are supporting a causal role of unrepaired oxidative DNA damage in the pathology of CS, a disorder showing neurological and aging features overlapping those present in TTD [82–84]. Conversely, hypersensitivity to oxidative stress remains elusive in TTD. Based on our current knowledge, it is likely that aging signs, as well as other clinical symptoms of TTD that are present also in the patients with normal repair ability, are directly related to transcriptional failure, which might be further exacerbated by the accumulation of DNA damage.

5.3. Lack of skin cancer

We do not have yet a clear answer why TTD patients, despite being mutated in the same genes as XP patients, do not show the increased freckle-like pigmentation and the high frequency of sunlight-induced skin cancers typical of XP. Following UV irradiation, the frequency of mutations show the same increase in NER-defective TTD and XP cells [50,85] and a similar failure in the checkpoint activation has been reported [86]. Only in XP-D fibroblasts from XP/CS patients, despite the fact that UV lesions are not processed by NER, the checkpoint is normally activated, likely by DNA breaks that occur at sites of transcription initiation. Differences between XP and TTD have been reported in immune responses, cell-cycle regulation, oxidative metabolism, apoptotic response (reviewed in [87]), gene expression after UV [88] and, more recently, behavior in the recruitment of NER factors to sites of localized UV damage [45,89]. Nevertheless, no cellular/biochemical alterations unequivocally correlated with the different cancer susceptibility in XP and TTD have been identified yet. At present, the difference in cancer proneness between XP and TTD, despite apparently similar repair defects, remains one of the most provocative enigmas in the field of DNA repair. A widely accepted explanation is that the defect in transcription, together with the limiting amounts of TFIIH typically detected in TTD cells, could prevent neoplastic transformation and/or progression by interfering with the expression of critical genes involved in the carcinogenic pathway [6,90,91]. As discussed in [6], this hypothesis implies a subtle interplay between the complex pathways of transcription, differentiation, and carcinogenesis. It would not be surprising if the outcome of this balance differed between species; therefore, the finding that the TTD mouse is, unlike TTD patients, susceptible to UVB-induced carcinogenesis [92] is not inconsistent with this hypothesis.

6. Future perspectives

Thus far, investigations of the mechanistic defects leading to XP or TTD have been beneficial in understanding the function of XPB, XPD, and p8/TTDA in NER and in transcription. Despite the notable progress made in our understanding of the genetic and molecular

bases of TTD, it is evident that there is still much more to learn about this disorder. Further studies are needed to elucidate the individual contribution of the deregulated genes and signalling pathways in the clinical outcome of TTD as well as their role in the acceleration of the aging process and in the suppression of skin tumorigenesis. Furthermore, we still do not know if and how the TFIIH complexes mutated in TTD interfere with the emerging role of TFIIH in transcription elongation and termination of some genes [93], and with the transcription of microRNAs whose expression in mammalian cells is driven by RNAPol II and it is highly regulated in a tissue and time-specific manner. Future research will have also to address the cloning of the other disease-genes responsible for TTD and the identification of their functions, a fundamental step toward developing therapeutic strategies.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

- [1] V.H. Price, R.B. Odom, W.H. Ward, F.T. Jones, Trichothiodystrophy: sulfur-deficient brittle hair as a marker for a neuroectodermal symptom complex, *Arch. Dermatol.* 116 (1980) 1375–1384.
- [2] E. Bergmann, J.M. Egly, Trichothiodystrophy, a transcription syndrome, *Trends Genet.* 17 (2001) 279–286.
- [3] S. Faghri, D. Tamura, K.H. Kraemer, J.J. Digiovanna, Trichothiodystrophy: a systematic review of 112 published cases characterises a wide spectrum of clinical manifestations, *J. Med. Genet.* 45 (2008) 609–621.
- [4] M. Stefanini, M. Ruggieri, Trichothiodystrophy, in: M. Ruggieri, I. Pascual-Castroviejo, C. Di Rocco (Eds.), *Neurocutaneous Diseases*, Springer-Verlag, New York Wien, 2008, pp. 821–845.
- [5] W.J. Kleijer, V. Laugel, M. Berneburg, T. Nardo, H. Fawcett, A. Gratchev, N.G. Jaspers, A. Sarasin, M. Stefanini, A.R. Lehmann, Incidence of DNA repair deficiency disorders in western Europe: Xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy, *DNA Repair (Amst.)* 7 (2008) 744–750.
- [6] A.R. Lehmann, The xeroderma pigmentosum group D (XPD) gene: one gene, two functions, three diseases, *Genes Dev.* 15 (2001) 15–23.
- [7] Y. Takagi, C.A. Masuda, W.H. Chang, H. Komori, D. Wang, T. Hunter, C.A. Joazeiro, R.D. Kornberg, Ubiquitin ligase activity of TFIIH and the transcriptional response to DNA damage, *Mol. Cell* 18 (2005) 237–243.
- [8] M. Zurita, C. Merino, The transcriptional complexity of the TFIIH complex, *Trends Genet.* 19 (2003) 578–584.
- [9] M. Stefanini, in: A.S. Balajee (Ed.), *Trichothiodystrophy: A Disorder Highlighting the Crosstalk Between DNA Repair and Transcription*, Landes Bioscience, 2006, pp. 30–46.
- [10] S. Ito, I. Kuraoka, P. Chymkowitz, E. Compe, A. Takedachi, C. Ishigami, F. Coin, J.M. Egly, K. Tanaka, XPG stabilizes TFIIH, allowing transactivation of nuclear receptors: implications for Cockayne syndrome in XP-G/CS patients, *Mol. Cell* 26 (2007) 231–243.
- [11] O.D. Scharer, Hot topics in DNA repair: the molecular basis for different disease states caused by mutations in TFIIH and XPG, *DNA Repair (Amst.)* 7 (2008) 339–344.
- [12] F. Coin, V. Oksenysh, V. Mocquet, S. Groh, C. Blattner, J.M. Egly, Nucleotide excision repair driven by the dissociation of CAK from TFIIH, *Mol. Cell* 31 (2008) 9–20.
- [13] V. Oksenysh, B.B. de Jesus, A. Zhovmer, J.M. Egly, F. Coin, Molecular insights into the recruitment of TFIIH to sites of DNA damage, *EMBO J.* 28 (2009) 2971–2980.
- [14] S. Larochele, J. Pandur, R.P. Fisher, H.K. Salz, B. Suter, Cdk7 is essential for mitosis and for in vivo Cdk-activating kinase activity, *Genes Dev.* 12 (1998) 370–381.
- [15] S. Larochele, K.A. Merrick, M.E. Terret, L. Wohlbold, N.M. Barboza, C. Zhang, K.M. Shokat, P.V. Jallepalli, R.P. Fisher, Requirements for Cdk7 in the assembly of Cdk1/cyclin B and activation of Cdk2 revealed by chemical genetics in human cells, *Mol. Cell* 25 (2007) 839–850.
- [16] S. Iben, H. Tschochner, M. Bier, D. Hoogstraten, P. Hozak, J.M. Egly, I. Grummt, TFIIH plays an essential role in RNA polymerase I transcription, *Cell* 109 (2002) 297–306.

- [17] D. Hoogstraten, A.L. Nigg, H. Heath, L.H. Mullenders, R. van Driel, J.H. Hoeijmakers, W. Vermeulen, A.B. Houtsmuller, Rapid switching of TFIIH between RNA polymerase I and II transcription and DNA repair in vivo, *Mol. Cell* 10 (2002) 1163–1174.
- [18] G. Giglia-Mari, A. Theil, P.O. Mari, S. Mourgues, J. Nonnekens, L.O. Andrieux, J. de Wit, C. Miquel, N. Wijgers, A. Maas, M. Foustier, J. Hoeijmakers, W. Vermeulen, Differentiation driven changes in the dynamic organization of basal transcription initiation, *PLoS Biol.* 7 (2009) e10000220.
- [19] J.M. Egly, The 14th Datta Lecture. TFIIH: from transcription to clinic, *FEBS Lett.* 498 (2001) 124–128.
- [20] F. Coin, V. Oksnych, J.M. Egly, Distinct roles for the XPB/p52 and XPD/p44 subcomplexes of TFIIH in damaged DNA opening during nucleotide excision repair, *Mol. Cell* 26 (2007) 245–256.
- [21] F. Tirode, D. Busso, F. Coin, J.M. Egly, Reconstitution of the transcription factor TFIIH: assignment of functions for the three enzymatic subunits, XPB, XPD, and cdk7, *Mol. Cell* 3 (1999) 87–95.
- [22] F. Coin, J.C. Marinoni, C. Rodolfo, S. Fribourg, A.M. Pedrini, J.M. Egly, Mutations in the XPD helicase gene result in XP and TTD phenotypes, preventing interaction between XPD and the p44 subunit of TFIIH, *Nat. Genet.* 20 (1998) 184–188.
- [23] B.M. Bernardes de Jesus, M. Bjoras, F. Coin, J.M. Egly, Dissection of the molecular defects caused by pathogenic mutations in the DNA repair factor XPC, *Mol. Cell Biol.* 28 (2008) 7225–7235.
- [24] M. Honda, J. Park, R.A. Pugh, T. Ha, M. Spies, Single-molecule analysis reveals differential effect of ssDNA-binding proteins on DNA translocation by XPD helicase, *Mol. Cell* 35 (2009) 694–703.
- [25] J.A. Ranish, S. Hahn, Y. Lu, E.C. Yi, X.J. Li, J. Eng, R. Aebersold, Identification of TFB5, a new component of general transcription and DNA repair factor IIH, *Nat. Genet.* 36 (2004) 707–713.
- [26] G. Giglia-Mari, F. Coin, J.A. Ranish, D. Hoogstraten, A. Theil, N. Wijgers, N.G. Jaspers, A. Raams, M. Argentini, P.J. van der Spek, E. Botta, M. Stefanini, J.M. Egly, R. Aebersold, J.H. Hoeijmakers, W. Vermeulen, A new, tenth subunit of TFIIH is responsible for the DNA repair syndrome trichothiodystrophy group A, *Nat. Genet.* 36 (2004) 714–719.
- [27] F. Coin, L. Proietti De Santis, T. Nardo, O. Zlobinskaya, M. Stefanini, J.M. Egly, p8/TTD-A as a repair-specific TFIIH subunit, *Mol. Cell* 21 (2006) 215–226.
- [28] G. Giglia-Mari, C. Miquel, A.F. Theil, P.O. Mari, D. Hoogstraten, J.M. Ng, C. Dinant, J.H. Hoeijmakers, W. Vermeulen, Dynamic interaction of TTD-A with TFIIH is stabilized by nucleotide excision repair in living cells, *PLoS Biol.* 4 (2006) e156.
- [29] M. Vitorino, F. Coin, O. Zlobinskaya, R.A. Atkinson, D. Moras, J.M. Egly, A. Poterszman, B. Kieffer, Solution structure and self-association properties of the p8 TFIIH subunit responsible for trichothiodystrophy, *J. Mol. Biol.* 368 (2007) 473–480.
- [30] D.E. Kainov, M. Vitorino, J. Cavarelli, A. Poterszman, J.M. Egly, Structural basis for group A trichothiodystrophy, *Nat. Struct. Mol. Biol.* 15 (2008) 980–984.
- [31] M. Stefanini, W. Vermeulen, G. Weeda, S. Giliani, T. Nardo, M. Mezzina, A. Sarasin, J.I. Harper, C.F. Arlett, J.H. Hoeijmakers, A.R. Lehmann, A new nucleotide-excision-repair gene associated with the disorder trichothiodystrophy, *Am. J. Hum. Genet.* 53 (1993) 817–821.
- [32] W. Vermeulen, E. Bergmann, J. Auriol, S. Rademakers, P. Frit, E. Appeldoorn, J.H. Hoeijmakers, J.M. Egly, Sublimiting concentration of TFIIH transcription/DNA repair factor causes TTD-A trichothiodystrophy disorder, *Nat. Genet.* 26 (2000) 307–313.
- [33] E. Botta, T. Nardo, A.R. Lehmann, J.M. Egly, A.M. Pedrini, M. Stefanini, Reduced level of the repair/transcription factor TFIIH in trichothiodystrophy, *Hum. Mol. Genet.* 11 (2002) 2919–2928.
- [34] G. Weeda, E. Eveno, I. Donker, W. Vermeulen, O. Chevallier-Lagente, A. Taieb, A. Stary, J.H. Hoeijmakers, M. Mezzina, A. Sarasin, A mutation in the XPB/ERCC3 DNA repair transcription gene, associated with trichothiodystrophy, *Am. J. Hum. Genet.* 60 (1997) 320–329.
- [35] J. Bradsher, F. Coin, J.M. Egly, Distinct roles for the helicases of TFIIH in transcript initiation and promoter escape, *J. Biol. Chem.* 275 (2000) 2532–2538.
- [36] D. Van Neste, Congenital hair dysplasia: management and value of various diagnostic methods, *Ann. Dermatol. Venereol.* 116 (1989) 251–263.
- [37] G. Weeda, R.C. van Ham, W. Vermeulen, D. Bootsma, A.J. van der Eb, J.H. Hoeijmakers, A presumed DNA helicase encoded by ERCC-3 is involved in the human repair disorders xeroderma pigmentosum and Cockayne's syndrome, *Cell* 62 (1990) 777–791.
- [38] K.S. Oh, S.G. Khan, N.G. Jaspers, A. Raams, T. Ueda, A. Lehmann, P.S. Friedmann, S. Emmert, A. Gratchev, K. Lachlan, A. Lucassan, C.C. Baker, K.H. Kraemer, Phenotypic heterogeneity in the XPB DNA helicase gene (ERCC3): xeroderma pigmentosum without and with Cockayne syndrome, *Hum. Mutat.* 27 (2006) 1092–1103.
- [39] B.C. Broughton, H. Steingrimsdottir, C.A. Weber, A.R. Lehmann, Mutations in the xeroderma pigmentosum group D DNA repair/transcription gene in patients with trichothiodystrophy, *Nat. Genet.* 7 (1994) 189–194.
- [40] K. Takayama, E.P. Salazar, B.C. Broughton, A.R. Lehmann, A. Sarasin, L.H. Thompson, C.A. Weber, Defects in the DNA repair and transcription gene ERCC2(XPD) in trichothiodystrophy, *Am. J. Hum. Genet.* 58 (1996) 263–270.
- [41] K. Takayama, D.M. Danks, E.P. Salazar, J.E. Cleaver, C.A. Weber, DNA repair characteristics and mutations in the ERCC2 DNA repair and transcription gene in a trichothiodystrophy patient, *Hum. Mutat.* 9 (1997) 519–525.
- [42] E.M. Taylor, B.C. Broughton, E. Botta, M. Stefanini, A. Sarasin, N.G. Jaspers, H. Fawcett, S.A. Harcourt, C.F. Arlett, A.R. Lehmann, Xeroderma pigmentosum and trichothiodystrophy are associated with different mutations in the XPD (ERCC2) repair/transcription gene, *Proc. Natl. Acad. Sci. U.S.A.* 94 (1997) 8658–8663.
- [43] E. Botta, T. Nardo, B.C. Broughton, S. Marinoni, A.R. Lehmann, M. Stefanini, Analysis of mutations in the XPD gene in Italian patients with trichothiodystrophy: site of mutation correlates with repair deficiency, but gene dosage appears to determine clinical severity, *Am. J. Hum. Genet.* 63 (1998) 1036–1048.
- [44] V. Viprakasit, R.J. Gibbons, B.C. Broughton, J.L. Tolmie, D. Brown, P. Lunt, R.M. Winter, S. Marinoni, M. Stefanini, L. Brueton, A.R. Lehmann, D.R. Higgs, Mutations in the general transcription factor TFIIH result in beta-thalassaemia in individuals with trichothiodystrophy, *Hum. Mol. Genet.* 10 (2001) 2797–2802.
- [45] J. Boyle, T. Ueda, K.S. Oh, K. Imoto, D. Tamura, J. Jagdeo, S.G. Khan, C. Nadem, J.J. Digiovanna, K.H. Kraemer, Persistence of repair proteins at unrepaired DNA damage distinguishes diseases with ERCC2 (XPD) mutations: cancer-prone xeroderma pigmentosum vs. non-cancer-prone trichothiodystrophy, *Hum. Mutat.* 29 (2008) 1194–1208.
- [46] E. Botta, T. Nardo, D. Orioli, R. Guglielmino, R. Ricotti, S. Bondanza, F. Benedicenti, G. Zambruno, M. Stefanini, Genotype-phenotype relationships in trichothiodystrophy patients with novel splicing mutations in the XPD gene, *Hum. Mutat.* 30 (2009) 438–445.
- [47] S. Dubaele, L. Proietti De Santis, R.J. Bienstock, A. Keriel, M. Stefanini, B. Van Houten, J.M. Egly, Basal transcription defect discriminates between xeroderma pigmentosum and trichothiodystrophy in XPD patients, *Mol. Cell* 11 (2003) 1635–1646.
- [48] M. Stefanini, K.H. Kraemer, Xeroderma pigmentosum, in: M. Ruggieri, I. Pascual-Castroviejo, C. Di Rocco (Eds.), *Neurocutaneous Diseases*, Springer-Verlag, New York Wien, 2008, pp. 771–792.
- [49] S. Emmert, T. Ueda, U. Zumsteg, P. Weber, S.G. Khan, K.S. Oh, J. Boyle, P. Laspe, K. Zachmann, L. Boeckmann, C. Kuschal, A. Bircher, K.H. Kraemer, Strict sun protection results in minimal skin changes in a patient with xeroderma pigmentosum and a novel c.2009delG mutation in XPD (ERCC2), *Exp. Dermatol.* 18 (2009) 64–68.
- [50] M. Stefanini, G. Orecchia, G. Rabbiosi, F. Nuzzo, Altered cellular response to UV irradiation in a patient affected by premature ageing, *Hum. Genet.* 73 (1986) 189–192.
- [51] A.R. Lehmann, C.F. Arlett, B.C. Broughton, S.A. Harcourt, H. Steingrimsdottir, M. Stefanini, A. Malcolm, R. Taylor, A.T. Natarajan, S. Green, M.D. King, R.M. MacKie, J.B.P. Stephenson, J.L. Tolmie, Trichothiodystrophy, a human DNA repair disorder with heterogeneity in the cellular response to ultraviolet light, *Cancer Res.* 48 (1988) 6090–6096.
- [52] M. Stefanini, S. Giliani, T. Nardo, S. Marinoni, V. Nazzaro, R. Rizzo, G. Trevisan, DNA repair investigations in nine Italian patients affected by trichothiodystrophy, *Mutat. Res.* 273 (1992) 119–125.
- [53] M. Stefanini, P. Lagomarsini, S. Giliani, T. Nardo, E. Botta, A. Peserico, W.J. Kleijer, A.R. Lehmann, A. Sarasin, Genetic heterogeneity of the excision repair defect associated with trichothiodystrophy, *Carcinogenesis* 14 (1993) 1101–1105.
- [54] B.C. Broughton, M. Berneburg, H. Fawcett, E.M. Taylor, C.F. Arlett, T. Nardo, M. Stefanini, E. Menefee, V.H. Price, S. Queille, A. Sarasin, E. Bohnert, J. Krutmann, R. Davidson, K.H. Kraemer, A.R. Lehmann, Two individuals with features of both xeroderma pigmentosum and trichothiodystrophy highlight the complexity of the clinical outcomes of mutations in the XPD gene, *Hum. Mol. Genet.* 10 (2001) 2539–2547.
- [55] J.O. Andressoo, J. Jans, J. de Wit, F. Coin, D. Hoogstraten, M. van de Ven, W. Toussaint, J. Huijman, H.B. Thio, W.J. van Leeuwen, J. de Boer, J.M. Egly, J.H. Hoeijmakers, G.T. van der Horst, J.R. Mitchell, Rescue of progeria in trichothiodystrophy by homozygous lethal Xpd alleles, *PLoS Biol.* 4 (2006) e322.
- [56] K. Nakabayashi, D. Amann, Y. Ren, U. Saarialho-Kere, N. Avidan, S. Gentles, J.R. MacDonald, E.G. Puffenberger, A.M. Christiano, A. Martinez-Mir, J.C. Salas-Alanis, R. Rizzo, E. Vamos, A. Raams, C. Les, E. Seboun, N.G. Jaspers, J.S. Beckmann, C.E. Jackson, S.W. Scherer, Identification of C7orf11 (TTDN1) gene mutations and genetic heterogeneity in nonphotosensitive trichothiodystrophy, *Am. J. Hum. Genet.* 76 (2005) 510–516.
- [57] E. Botta, J. Offman, T. Nardo, R. Ricotti, G. Zambruno, D. Sansone, P. Balestri, A. Raams, W.J. Kleijer, N.G. Jaspers, A. Sarasin, A.R. Lehmann, M. Stefanini, Mutations in the C7orf11 (TTDN1) gene in six nonphotosensitive trichothiodystrophy patients: no obvious genotype-phenotype relationships, *Hum. Mutat.* 28 (2007) 92–96.
- [58] Y. Zhang, Y. Tian, Q. Chen, D. Chen, Z. Zhai, H.B. Shu, TTDN1 is a Plk1-interacting protein involved in maintenance of cell cycle integrity, *Cell. Mol. Life Sci.* 64 (2007) 632–640.
- [59] M.A. Kleinschmidt, G. Streubel, B. Samans, M. Krause, U.M. Bauer, The protein arginine methyltransferases CARM1 and PRMT1 cooperate in gene regulation, *Nucleic Acids Res.* 36 (2008) 3202–3213.
- [60] W. Vermeulen, S. Rademakers, N.G. Jaspers, E. Appeldoorn, A. Raams, B. Klein, W.J. Kleijer, L.K. Hansen, J.H. Hoeijmakers, A temperature-sensitive disorder in basal transcription and DNA repair in humans, *Nat. Genet.* 27 (2001) 299–303.
- [61] J. de Boer, J. de Wit, H. van Steeg, R.J. Berg, H. Morreau, P. Visser, A.R. Lehmann, M. Duran, J.H. Hoeijmakers, G. Weeda, A mouse model for the basal transcription/DNA repair syndrome trichothiodystrophy, *Mol. Cell* 1 (1998) 981–990.
- [62] J. de Boer, J.O. Andressoo, J. de Wit, J. Huijman, R.B. Beems, H. van Steeg, G. Weeda, G.T. van der Horst, W. van Leeuwen, A.P. Themmen, M. Meradji, J.H. Hoeijmakers, Premature aging in mice deficient in DNA repair and transcription, *Science* 296 (2002) 1276–1279.
- [63] J. de Boer, I. Donker, J. de Wit, J.H. Hoeijmakers, G. Weeda, Disruption of the mouse xeroderma pigmentosum group D DNA repair/basal transcription gene results in preimplantation lethality, *Cancer Res.* 58 (1998) 89–94.
- [64] L. Fan, J.O. Fuss, Q.J. Cheng, A.S. Arvai, M. Hammel, V.A. Roberts, P.K. Cooper, J.A. Tainer, XPD helicase structures and activities: insights into the cancer and aging phenotypes from XPD mutations, *Cell* 133 (2008) 789–800.

- [65] H. Liu, J. Rudolf, K.A. Johnson, S.A. McMahon, M. Oke, L. Carter, A.M. McRobbie, S.E. Brown, J.H. Naismith, M.F. White, Structure of the DNA repair helicase XPD, *Cell* 133 (2008) 801–812.
- [66] S.C. Wolski, J. Kuper, P. Hanzelmann, J.J. Truglio, D.L. Croteau, B. Van Houten, C. Kisker, Crystal structure of the FeS cluster-containing nucleotide excision repair helicase XPD, *PLoS Biol.* 6 (2008) e149.
- [67] A.R. Lehmann, XPD structure reveals its secrets, *DNA Repair (Amst.)* 7 (2008) 1912–1915.
- [68] L. Racioppi, C. Cancrini, M.L. Romiti, F. Angelini, S. Di Cesare, E. Bertini, S. Livi-diotti, M.G. Gambarara, G. Matarese, F. Lago Paz, M. Stefanini, P. Rossi, Defective dendritic cell maturation in a child with nucleotide excision repair deficiency and CD4 lymphopenia, *Clin. Exp. Immunol.* 126 (2001) 511–518.
- [69] C. Backendorf, J. de Wit, M. van Oosten, G.J. Stout, J.R. Mitchell, A.M. Borgstein, G.T. van der Horst, F.R. de Gruijl, J. Brouwer, L.H. Mullenders, J.H. Hoeijmakers, Repair characteristics and differentiation propensity of long-term cultures of epidermal keratinocytes derived from normal and NER-deficient mice, *DNA Repair (Amst.)* 4 (2005) 1325–1336.
- [70] A. Keriél, A. Sary, A. Sarasin, C. Rochette-Egly, J.M. Egly, XPD mutations prevent TFIIH-dependent transactivation by nuclear receptors and phosphorylation of RARalpha, *Cell* 109 (2002) 125–135.
- [71] E. Compe, P. Drane, C. Laurent, K. Diderich, C. Braun, J.H. Hoeijmakers, J.M. Egly, Dysregulation of the peroxisome proliferator-activated receptor target genes by XPD mutations, *Mol. Cell Biol.* 25 (2005) 6065–6076.
- [72] E. Compe, M. Malerba, L. Soler, J. Marescaux, E. Borrelli, J.M. Egly, Neurological defects in trichothiodystrophy reveal a coactivator function of TFIIH, *Nat. Neurosci.* 10 (2007) 1414–1422.
- [73] A. Lehmann, Ageing: repair and transcription keep us from premature ageing, *Curr. Biol.* 12 (2002) R550–551.
- [74] J. de Boer, J.H. Hoeijmakers, Cancer from the outside, aging from the inside: mouse models to study the consequences of defective nucleotide excision repair, *Biochimie* 81 (1999) 127–137.
- [75] A.A. van Apeldoorn, J. de Boer, H. van Steeg, J.H. Hoeijmakers, C. Otto, C.A. van Blitterswijk, Physicochemical composition of osteoporotic bone in the trichothiodystrophy premature aging mouse determined by confocal Raman microscopy, *J. Gerontol. A Biol. Sci. Med. Sci.* 62 (2007) 34–40.
- [76] D.J. Rossi, D. Bryder, J. Seita, A. Nussenzweig, J. Hoeijmakers, I.L. Weissman, Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age, *Nature* 447 (2007) 725–729.
- [77] S.W. Wijnhoven, R.B. Beems, M. Roodbergen, J. van den Berg, P.H. Lohman, K. Diderich, G.T. van der Horst, J. Vijg, J.H. Hoeijmakers, H. van Steeg, Accelerated aging pathology in ad libitum fed Xpd(TTD) mice is accompanied by features suggestive of caloric restriction, *DNA Repair (Amst.)* 4 (2005) 1314–1324.
- [78] G.A. Garinis, L.M. Uittenboogaard, H. Stachelscheid, M. Fousteri, W. van Ijcken, T.M. Breit, H. van Steeg, L.H. Mullenders, G.T. van der Horst, J.C. Bruning, C.M. Niessen, J.H. Hoeijmakers, B. Schumacher, Persistent transcription-blocking DNA lesions trigger somatic growth attenuation associated with longevity, *Nat. Cell Biol.* 11 (2009) 604–615.
- [79] B. Schumacher, G.A. Garinis, J.H. Hoeijmakers, Age to survive: DNA damage and aging, *Trends Genet.* 24 (2008) 77–85.
- [80] G.A. Garinis, G.T. van der Horst, J. Vijg, J.H. Hoeijmakers, DNA damage and ageing: new-age ideas for an age-old problem, *Nat. Cell Biol.* 10 (2008) 1241–1247.
- [81] J.Y. Park, M.O. Cho, S. Leonard, B. Calder, I.S. Mian, W.H. Kim, S. Wijnhoven, H. van Steeg, J. Mitchell, G.T. van der Horst, J. Hoeijmakers, P. Cohen, J. Vijg, Y. Suh, Homeostatic imbalance between apoptosis and cell renewal in the liver of premature aging Xpd mice, *PLoS One* 3 (2008) e2346.
- [82] M. D'Errico, E. Parlanti, M. Teson, P. Degan, T. Lemma, A. Calcagnini, I. Iavarone, P. Jaruga, M. Ropolo, A.M. Pedrini, D. Orioli, G. Frosina, G. Zambruno, M. Diz-daroglu, M. Stefanini, E. Dogliotti, The role of CSA in the response to oxidative DNA damage in human cells, *Oncogene* 26 (2007) 4336–4343.
- [83] T. Stevnsner, M. Muftuoglu, M.D. Aamann, V.A. Bohr, The role of Cockayne Syndrome group B (CSB) protein in base excision repair and aging, *Mech. Ageing Dev.* 129 (2008) 441–448.
- [84] T. Nardo, R. Oneda, G. Spivak, B. Vaz, L. Mortier, P. Thomas, D. Orioli, V. Laugel, A. Sary, P.C. Hanawalt, A. Sarasin, M. Stefanini, A UV-sensitive syndrome patient with a specific CSA mutation reveals separable roles for CSA in response to UV and oxidative DNA damage, *Proc. Natl. Acad. Sci. U.S.A.* 106 (2009) 6209–6214.
- [85] C. Madzak, J. Armier, A. Sary, L. Daya-Grosjean, A. Sarasin, UV-induced mutations in a shuttle vector replicated in repair deficient trichothiodystrophy cells differ with those in genetically related cancer prone xeroderma pigmentosum, *Carcinogenesis* 14 (1993) 1255–1260.
- [86] F. Marini, T. Nardo, M. Giannattasio, M. Minuzzo, M. Stefanini, P. Plevani, M. Muzi Falconi, DNA nucleotide excision repair-dependent signaling to checkpoint activation, *Proc. Natl. Acad. Sci. U.S.A.* 103 (2006) 17325–17330.
- [87] A.R. Lehmann, DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy, *Biochimie* 85 (2003) 1101–1111.
- [88] R.M. da Costa, L. Riou, A. Paquola, C.F. Menck, A. Sarasin, Transcriptional profiles of unirradiated or UV-irradiated human cells expressing either the cancer-prone XPB/CS allele or the noncancer-prone XPB/TTD allele, *Oncogene* 24 (2005) 1359–1374.
- [89] V. Chigancas, K.M. Lima-Bessa, A. Sary, C.F. Menck, A. Sarasin, Defective transcription/repair factor IIH recruitment to specific UV lesions in trichothiodystrophy syndrome, *Cancer Res.* 68 (2008) 6074–6083.
- [90] D. Bootsma, J.H. Hoeijmakers, DNA repair. Engagement with transcription, *Nature* 363 (1993) 114–115.
- [91] M. Berneburg, P.H. Clingen, S.A. Harcourt, J.E. Lowe, E.M. Taylor, M.H. Green, J. Krutmann, C.F. Arlett, A.R. Lehmann, The cancer-free phenotype in trichothiodystrophy is unrelated to its repair defect, *Cancer Res.* 60 (2000) 431–438.
- [92] J. de Boer, H. van Steeg, R.J. Berg, J. Garssen, J. de Wit, C.T. van Oostrum, R.B. Beems, G.T. van der Horst, C.F. van Kreijl, F.R. de Gruijl, D. Bootsma, J.H. Hoeijmakers, G. Weeda, Mouse model for the DNA repair/basal transcription disorder trichothiodystrophy reveals cancer predisposition, *Cancer Res.* 59 (1999) 3489–3494.
- [93] K. Glover-Cutter, S. Larochele, B. Erickson, C. Zhang, K. Shokat, R.P. Fisher, D.L. Bentley, TFIIH-associated Cdk7 kinase functions in phosphorylation of CTD Ser7 residues, promoter-proximal pausing, and termination by RNA polymerase II, *Mol. Cell Biol.* 29 (2009) 5455–5464.