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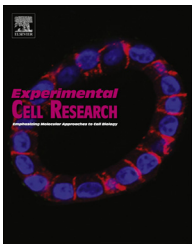

Review Article

TTDA: Big impact of a small protein

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ABSTRACT

Nucleotide excision repair (NER) is a highly versatile DNA repair process which is able to remove a broad spectrum of structurally unrelated DNA helix-destabilizing lesions. The multi-subunit transcription/repair factor IIH (TFIIH) is an important decision maker in NER, by opening the DNA double helix after the initial damage recognition and subsequently verifying the lesion. Inherited mutations in TFIIH subunits are associated with NER-deficiency and a perplexing clinical heterogeneity, ranging from cancer-prone Xeroderma Pigmentosum to the progeroid diseases Cockayne Syndrome and Trichothiodystrophy (TTD). Three different TFIIH coding genes are implicated in TTD: *XPD*, *XPB* and *TTDA*. The latter gene encodes for a small (71 amino-acid) subunit and appeared important for the stabilization of the entire TFIIH complex. Based on analyzing TTD group A patient derived cells it was initially thought that TTDA has only a NER-stimulating role. In this review we summarize recent data showing that full disruption of *TTDA* expression in a knock-out mouse-model completely inactivates NER. Surprisingly, next to being essential for NER, TTDA appeared to be required also for embryonic development, indicative for the big impact this small protein has on basal biological processes.

Contents

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Introduction

Nucleotide excision repair (NER) is a highly coordinated multistep pathway that removes a remarkable wide range of DNA lesions that disturb the Watson and Crick base pairing [1], including the UV-light induced photoproducts cyclobutane pyrimine dimers (CPDs) and 6-4 pyrimidine pyrimidone photoproducts (6-4PPs), and various bulky chemical adducts. This mechanism consists of two DNA damage sensing sub-pathways: one that scans the entire genome for lesions (global genome NER, GG-NER) and one that targets lesions that physically block the elongating RNA polymerase II (transcription-coupled NER, TC-NER). After these two modes of lesion recognition the dual functional repair/transcription factor IIH (TFIIH) is recruited. TFIIH is a multi-subunit complex that was originally identified as an essential transcription initiation factor, but also appeared pivotal for NER [2,3]. In both processes TFIIH functions to open the DNA double helix, catalyzed by its two helicase subunits XPB and XPD [2,4]. TFIIH is composed of ten subunits, seven of which (XPB, XPD, p62, p52, p44, p34 and TTDA) form the core complex and an associated heterotrimeric Cdk-activating kinase (CAK, consisting of CDK7, CCNH and MAT1) [5,6]. TFIIH further functions in lesion verification, interacts with the downstream NER factors XPA and XPG. These factors together with RPA recruit and properly orient the ERCC1-XPF endonuclease, which incises 5′ to the lesion followed by the 3′ incision by XPG. The resulting 22–30 nucleotide gap is filled by the replication machinery [1].

Next to essential functions in transcription initiation and NER, TFIIH is also implicated in transcription of ribosomal genes by RNA polymerase I, and basal and activated transcription by RNA polymerase II [5,6]. The importance of a fully functional TFIIH is clearly illustrated by the severe clinical consequences associated with inherited defects in this complex. The UV-hypersensitive human NER syndromes Xeroderma Pigmentosum (XP), Cockayne Syndrome (CS), combined XP and CS (XP/CS), Trichothiodystrophy (TTD) or combined XP and TTD (XP/TTD) [7–10] are associated with a strongly enhanced cancer-risk and/or dramatically accelerated segmental ageing. Mutations that affect only the GG-NER pathway mainly cause XP features, as non-repaired lesions induced by e.g. exposure to UV-light can trigger cell killing or can be converted into mutations. The former explains the severe sun-sensitivity, whereas increased mutagenesis in oncogenes and tumor-suppressor genes may explain the strong skin-cancer predisposition in XP. Contrary to GG-NER, mutations that affect the TC-NER pathway do not significantly contribute to mutagenesis, as this process deals only with a small fraction of total lesions, explaining at least in part the absence of elevated cancer in CS and TTD. However, lesion-blocked RNA polymerases—when unresolved—are extremely cytotoxic and mainly induce cell killing or senescence, which likely explains the segmental ageing features seen in CS and TTD [11]. Moreover, the high damage sensitivity due to the TC-NER defect causes cells to die, which also contributes to the reduced cancer risk of TC-NER disorders. Both cancer-related and age-related diseases/discomforts have become a major personal, social, medical and economical issue due to the still increasing life expectancy in developed countries worldwide. It is therefore highly relevant to understand the molecular mechanisms that cause these phenomena.

Trichothiodystrophy

The human syndrome Trichothiodystrophy (TTD) is a very rare, autosomal recessive disorder that is characterized by a low content of sulfur-rich proteins in both hair and nails and in addition many other neurodevelopmental features. The Greek term Trichothiodystrophy (tricho-thio-dys-trophe meaning hair-sulfur-faulty-nourishment) was therefore used to describe this group of patients based on the low-sulfur brittle hair and nails. The clinical symptoms observed in TTD patients are very variable in expression and severity, and display a broad spectrum of phenotypes including: photosensitivity, ichthyosis, brittle hair and nails, intellectual impairment, decreased fertility and short stature (the acronyms, PIBIDS, IBIDS and BIDS represent the initials of these characterizations [12]). The spectrum of symptoms observed in TTD patients stretches from very mild forms of the disease, characterized by normal development with only brittle hair and scaling skin (typical TTD-features) to very severe cases, characterized by high mortality at young age combined with severe neurodevelopmental defects.

Hitherto four genes have been identified that are causative for the TTD phenotype, namely *TTDN1*, *XPB*, *XPD*, and *TTDA*. Mutations in the *TTDN1* gene are associated with the non-photosensitive form of TTD [13,14]. However, the majority of non-photosensitive TTD patients (approximately 80%) has not yet been linked with any causative gene. In contrast, all photosensitive TTD patients have been found to carry a mutation in the *XPB*, *XPD* or *TTDA* genes [9] all encoding subunits of TFIIH and carrying a defect in NER, explaining the photosensitivity. Mutations in the XPB and XPD subunits of TFIIH induce a broad spectrum of clinical features including XP, CS and TTD; however, mutations in the TTDA subunit are thus far only found to be associated with TTD-specific symptoms. These patients belong to the rare TTD group A (TTD-A) and display a relatively mild TTD phenotype [15]. This review is aimed to better understand the etiology of the spectrum of symptoms presented in photosensitive TTD-A patients. Recently published data has provided new insights in TTDA's function, which are discussed below.

Characterization of TTDA

Despite the notion that NER-deficient TTD comprises three distinct genes [16,17] and that their respective encoded proteins are associated with TFIIH [17], the identity of the TTDA protein has remained enigmatic for long [18]. Within a proteomic screen of yeast RNA polymerase II transcription pre-initiation complexes a novel 72 amino acid containing TFIIH subunit (designated TFB5) was identified [19]. Due to its small size, the yeast protein and the human ortholog were previously overlooked in highly purified TFIIH complexes, but the human gene encoding the *TFB5* ortholog turned out to be the causative gene for TTD-A [15].

Thus far, only three *TTDA* mutations were identified in three non-related TTD-A patients: TTD99RO, TTD1BR and siblings TTD13PV and TTD14PV (amino acid changes in TTD-A patients proteins [15] are schematically depicted in Fig. 1). Interestingly, despite the rather diverse *TTDA* mutations, which are all expressed at mRNA level, the clinical features observed in TTD-A patients are quite similar [15]. Human TTDA is a very

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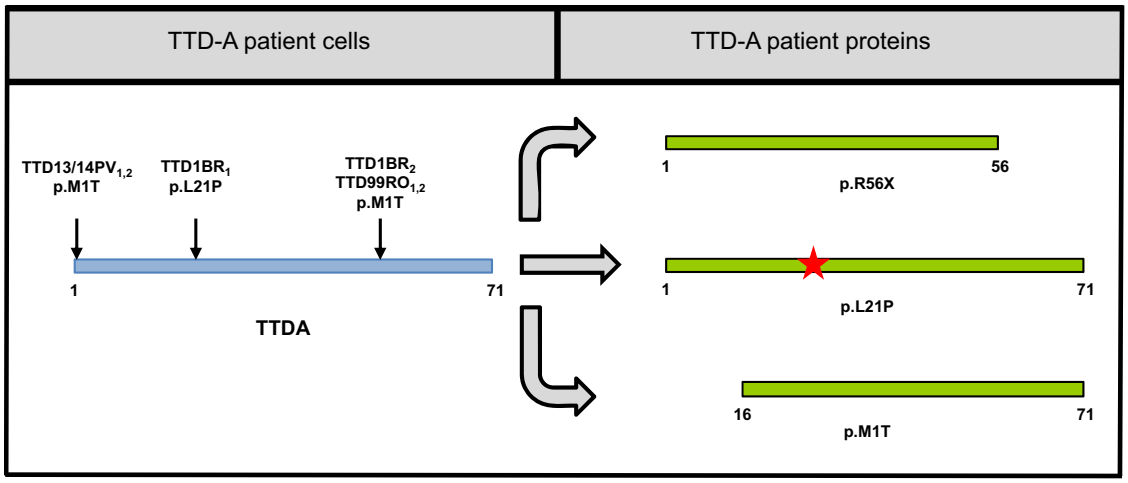

Fig. 1 – Amino acid changes in TTD-A patients proteins. (Left panel) Illustration of the TTDA protein (consisting of 71 amino acids) and the amino acid changes described in TTD-A patients, indicated with arrows. Patient TTD99RO: homozygous nonsense mutation at codon 56, converting Arginine to a stop codon (p.R56X). Patient TTD1BR: heterozygous for the TTD99RO allele, the other allele has a mis-sense mutation at codon 21, converting a conserved Leucine into Proline (p.L21P). Siblings TTD13PV and TTD14PV carry a homozygous mutation in the ATG start codon (p.M1T). The numbers 1 and 2 after the patient code represents the different alleles. (Right panel) Scheme of the expressed mutant proteins in the different TTD-A patient cells.

small, highly conserved, 8 kDa protein comprised of only 71 amino acids. There is no indication of any enzymatic activity or of a functional domain; however, it has preserved remarkable stretches of hydrophobic residues in almost all eukaryotes from human to yeast [15,19,20]. *In vitro* studies have revealed that TTDA binds to the TFIIH subunits XPD and p52. Particularly its interaction with p52, with whom it forms a heterodimer, improves TFIIH stability and regulates the ATPase activity of XPB during active NER [21,22]. Overexpressing TTDA in a DNA repair deficient human XP-D cells [22] or in a p52 Drosophila melanogaster mutant (Dmp52, which exhibits TTD and cancer-like phenotypes in the fly [23]), partly restored TFIIH levels and/or NER defects. Finally, a novel tripartite split-GFP system confirmed binding of TTDA to p52 in living human cells [24].

To better understand the molecular mechanism underlying the clinical symptoms presented by TTD patients, several *in vivo* experiments were performed to analyze cellular features associated with inherited mutations in the *TTDA* gene. TTDA analysis in living cells showed that green-fluorescent protein (GFP)-tagged TTDA is localized in both the cytoplasm and the nucleus, contrasting the strict nuclear localization of XPB-GFP [25]. Photo-bleaching experiments also indicated that the majority of TTDA is not associated to TFIIH and that only a small fraction at any time is bound to TFIIH. Although TTDA can be considered as integral component of TFIIH in biochemical terms [15], the association of TTDA-GFP to TFIIH (and to a lesser extent also of XPD-GFP) is much more dynamic than for example XPB-GFP [25]. However, DNA damage induction by UV increases the interaction of TTDA to TFIIH, suggesting a specific function of TTDA in NER [25]. Despite the notion that TTDA transiently interacts with TFIIH, this interaction is important to stabilize TFIIH and to sustain normal cellular TFIIH concentration. Solution structure analysis of purified recombinant human TTDA revealed that the isolated polypeptide homodimerizes, predominantly through antiparallel dimerization of the first N-terminal β-sheet [20]. This N-terminal region is also important for the interaction with p52 and XPD [22]. It is possible that the dimeric form of TTDA provides stability to non-TFIIH-associated protein [20] and that the dynamic equilibrium between free dimeric and TFIIH-bound TTDA provides TFIIH stabilizing property of TTDA.

Reduced steady-state levels of TFIIH in TTD-A cultured fibroblasts appeared to be a critical determinant in NER efficiency [18], but this lower concentration of the crucial basal transcription initiation factor in cultured cells does not seem to significantly affect the transcriptional competence. This suggests that NER requires higher concentrations of TFIIH and/or that the altered structure of the complex in the absence of TTDA mainly affects the NER function rather than the transcription role. Previous live cell kinetic studies [26] revealed that a relatively large proportion of the resident TFIIH molecules are recruited to NER sites at which they are bound significantly longer (4–5 min) than when bound for an average transcription initiation event (2–10 s). This difference in kinetic behavior of TFIIH when engaged in either transcription or DNA repair provides an explanation why one process appears more sensitive to relative enzyme concentrations than the other. The *in vitro* NER studies [22] showed that not only the concentration but also the composition of TFIIH is critical for its function in NER. In the absence of TTDA, only low levels of repair are detected, suggesting that association of TTDA to TFIIH renders this complex more competent for NER, at least *in vitro*.

NER in TTD-A patient cells

The cellular concentration of TFIIH in TTD-A fibroblasts is reduced to only 30% of the amount found in wild-type cells. TTD-A cells exhibit a very low DNA repair activity as determined by analyzing the NER-dependent repair replication in unscheduled DNA synthesis, when measured shortly after inducing a high dose of UV-light [15,18]. This low level of NER activity should render cells hypersensitive to UV. Strikingly, however, in UV-colony survival assays, measuring the long term biological effect of UV-irradiation, TTD-A

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cells appeared only moderately UV-sensitive [15]. Further analysis of different NER properties in TTD-A mutant cells revealed that the repair activity in TTD-A fibroblasts is not completely absent, but is most likely only slower [27]. This relative slow repair is on the long run able to remove most lesions, thus explaining the moderate UV-sensitivity. Indeed, UV-induced lesions that are mainly responsible for cytotoxicity (6-4PP) [28] are gradually removed in TTD-A cells compared to wild type cells contrary to what is observed in complete NER-deficient cells [27,28].

Ttda knock-out mice

In order to provide clues on the molecular basis of TTD-A related clinical features, we decided to generate a *Ttda*$^{-/-}$ mouse model [29]. Based on the severe mutations observed in the TTD-A patients, including truncation and start-codon mutations we opted for a complete inactivation of the gene. Unexpectedly, and in striking contrast to TTD-A patients, embryos that lack Ttda die between embryonic day 10.5 and just before birth. These embryos show a delay in development and have a reduction in size and body-weight. On top of these common features, *Ttda*$^{-/-}$ embryos also exhibit a rather heterogeneous spectrum of phenotypes. These additional features range from a “pale” appearance to internal hemorrhages, most likely leading to embryonic lethality (unpublished data). Using next generation sequencing we determined which genes were differentially expressed at a very early stage of gestation (unpublished data). Indeed, when we compared the transcriptional profile of embryonic stem cells (ES) isolated from *Ttda*$^{-/-}$ blastocysts with their wild type littermates, we found multiple genes being differentially expressed. Ingenuity Pathway Analysis highlighted several networks differentially regulated, including embryonic development, cellular growth and proliferation, and hematological system development and function. The TTD mouse model also showed that reduced transcription of specific genes in terminally differentiated skin keratinocytes explains the hallmark feature of TTD, brittle hair. From this model it is predicted that the reduced stability of TFIIH by TTD mutations affects transcription in other highly specialized or terminally differentiated cells [30]. The unexpected lethality of *Ttda*$^{-/-}$ embryos obviously does not allow a detailed organismal analysis to the nature of the TTD etiology. However, early embryonic development was still possible that allowed the isolation of embryonic cells.

Surprisingly, cells isolated from these mice present a complete inactivation of NER, which is in contrast to the relatively mild NER defect observed in human TTD-A cells. These striking differences of complete versus partial NER-deficiency and *in utero* lethality versus relative mild TTD symptoms between respectively, *Ttda*$^{-/-}$ mice and TTD-A patients, might be explained by the presence of partly functional mutant TTDA protein in patient cells. Unfortunately, the presence of low amounts of these mutant proteins cannot be confirmed, due to the lack of proper antibodies. However, expression of all TTD-A derived mutant proteins indeed partially corrected the complete absence of NER of *Ttda*$^{-/-}$ mouse cells [29]. Conversely, silencing of the mutant TTDA gene in the cells of TTD-A patients further reduced NER and UV survival, indicating residual activity of the mutant allele. The presence of a partly functional protein in TTD13/14PV patient is particularly intriguing, due to the homozygous translational start-site mutation [15]. Despite this ATG mutation, aberrant TTDA proteins are likely still being produced by initiating from an in-frame downstream ATG (codon 16) and produces low levels of an N-terminally truncated TTDA protein. Interestingly, it has been shown (both in yeast and humans) that the N-terminal domain of TTDA is important for binding to the TFIIH subunits XPD and p52 [22,31] and for its role in stimulating the ATPase-activity of the XPB subunit [22]. Furthermore, this interaction is also critical for the TFIIH stability. Apparently, the truncated TTDA protein is nevertheless able to carry out part of its function to permit residual NER. A possibility is that binding of TTDA, including the mutant variants, to TFIIH triggers a conformational change that increases the residence time of TFIIH on NER-lesions which facilitate the recruitment of downstream NER factors. The hypothesis of improperly structured TFIIH, due to mutated TTDA (or its complete absence) also fits with the observation that a lack of TTDA makes TFIIH less stable, as improperly folded proteins are usually vulnerable to degradation. Interaction with TTDA might stabilize the complex either by aiding folding (as a chaperone-like function) or by maintaining tertiary structure. A model comparing the recruitment of early NER factors in TTD-A patients and *Ttda*$^{-/-}$ mouse cells is depicted in Fig. 2. This model unites reduced repair with instability.

TTDA and oxidative DNA repair

TTDA's essential role in NER has far-reaching biological significance. However, the complete NER deficiency cannot explain the embryonic lethality, since other mice with fully compromised NER function (e.g. *Xpa*$^{-/-}$ mice) do not display similar developmental abnormalities and are fully viable [32,33]. Surprisingly, both ES cells and mouse embryonic fibroblasts derived from the *Ttda*$^{-/-}$ mice exhibit next to a complete NER defect, also hypersensitivity to several oxidizing agents, unlike other NER-deficient ES cells (e.g. *Xpa*$^{-/-}$), but similar as *Csb*$^{-/-}$ ES cells [34]. Based on these results, we suggest that *Ttda*$^{-/-}$ embryos are confronted with unrepaired endogenous oxidative lesions, possibly generated by low but continuous exposure to reactive oxygen species (ROS) and other natural reactive species during development. This compromised repair of oxidative DNA lesions may contribute to the lethal phenotype observed in the *Ttda*$^{-/-}$ embryos. Defects in multiple DNA repair systems may cause synergistic effects or even synthetic lethality [35,36]. Detailed analysis of different TFIIH mouse mutants revealed a correlation between sensitivity to oxidative DNA damage (endogenous DNA damage) and severity of the phenotype of the different models [34,37]. For example, severe developmental and premature aging problems have been seen in KO mouse models of DNA repair factors that function in independent repair pathways, such as *Xpa*$^{-/-}$ *Csb*$^{m/m}$ double mutant mice (defective in NER and TC-repair, including oxidative lesions) [38]. Importantly, this reduced resistance to oxidative DNA damage is likely not caused by a general (core) BER defect [29]. Previously, it was suggested that TFIIH is implicated in coordinating incision of lesion-stalled transcription complexes [39] and that some oxidative DNA lesions are processed by transcription-coupled repair [40,41]. It is also possible that some endogenously produced lesions or repair intermediates cause stalled RNA polymerase complexes, hindering alternative repair

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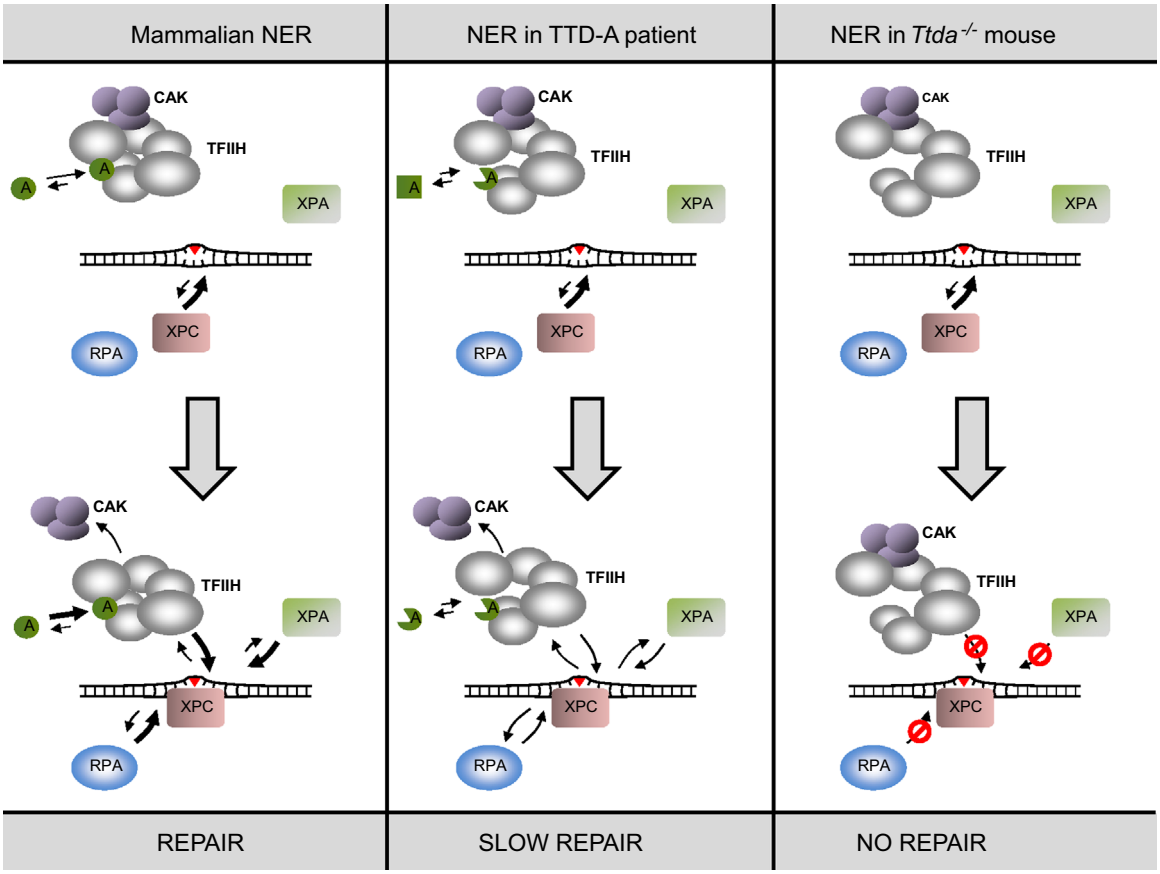

Fig. 2 – Proposed model for lesion-bound NER complex formation in mammalian cells, TTD-A patient cells and *Ttda*$^{-/-}$ cells. (Left panel) In mammalian wild type cells, damage sensor XPC stably binds to UV-induced DNA lesions, independently of downstream NER factors. After damage recognition, TFIIH opens the DNA helix and verifies the lesion, resulting in the release of the CAK complex. The accumulation of TFIIH shifts the equilibrium from a dissociated form of TTDA towards the bound state. After DNA unwinding, other pre-incision proteins such as RPA and XPA also accumulate at the damaged site, further stabilizing the NER complex and preparing for dual incision. (Middle panel) In TTD-A patient cells, damage sensor XPC stably binds to UV-induced DNA lesions, similar as occurs in wild-type cells. Mutant TTDA protein is not able to maintain TFIIH stability and conformation, resulting in a 70% reduction in protein concentration in the cell. Efficient binding of mutated TFIIH to the damaged site is also affected and reduces its affinity to the NER complex, diminishing the accumulation of downstream NER factors. NER complex assembly and the removal of the DNA lesions will take place, although in a kinetically slow manner. (Right panel) In *Ttda*$^{-/-}$ cells, damage sensor XPC stably binds to UV-induced DNA lesions, like in wild-type cells. Due to the absence of Ttda proteins, the TFIIH stability and conformation is affected, comparable to TTD-A patient cells. Without the presence of Ttda, TFIIH and also other downstream NER factors are unable to stably bind to DNA lesions. The complex assembly is completely abolished and as a result there is no repair.

pathways like GG-NER and BER to repair these stalling lesions [42,52].

TTDA and transcription

It is surprising to note that viability does not critically depend on TTDA and on high amounts of TFIIH, as embryos develop almost to birth. This is in striking contrast with deletion mutants of other tested TFIIH subunits, which are not viable in yeast and mammals. Incompatibility with live associated with deletions of TFIIH encoding genes was explained by the vital transcriptional role of TFIIH [43]. Extrapolation of this hypothesis thus argues that TTDA is not essential for the transcription reaction. Indeed TTDA appeared not to stimulate transcription *in vitro*, whereas it was shown to aid NER [22]. However, part of the non-NER-related features observed in TTD patients, such as brittle hair, are thought to be derived from an affected transcription function, due to the TTD-causing TFIIH mutations [30,44]. This apparent contradiction of the requirement of TTDA for transcription between *in vitro* experiments and observations in mouse tissue can be explained by the differential transcriptional program of specific tissues and cell types that are involved in TTD pathology. We propose a model in which transcription in terminally differentiated keratinocytes that form hair is limited to a group of cysteine-rich matrix protein genes. These gene products cross-link keratin filaments and provide strength to hair. Due to the highly specialized nature (terminal differentiation) of these cells the majority of the genome is transcriptionally repressed, including genes coding for house-keeping transcription factors. It is likely that in these

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cells transcription of TFIIH-encoding genes is also repressed and that transcription of the cysteine-rich matrix protein genes thus relies on the resident transcription factors. However, in TTD patient keratinocytes, due to the reduced TFIIH stability, the amount of TFIIH is not sufficient to support transcription of this group of genes by the time these cells have reached the final differentiation stage. Thus, reduced transcription of genes encoding for keratin filament crosslinking proteins that are the last to be expressed likely provides an explanation for the hallmark feature of TTD, brittle hair. Evidence for this hypothesis was provided by the observed reduced expression of the *SPRR2* gene in dorsal skin isolated from *TTD* mice, carrying a mutation in the *Xpd* gene, which mimics a mutation found in XP group D TTD patients [30]. *SPRR2* encodes for a structural component of the cornified envelope and is predominantly expressed in terminally differentiated keratinocytes. From this model it is predicted that the reduced stability of TFIIH by TTD mutations will affect transcription in other highly specialized or terminally differentiated cells. Indeed anemic features have been described in some TTD patients, likely due to reduced beta-globin expression [45]. Also precursor erythrocytes (just prior to nuclear eviction) are highly specialized in producing hemoglobin and predominantly abundantly transcribe globin genes. This observation further corroborates our hypothesis. In addition to the sub-limiting TFIIH concentrations, also the specific spatio-temporal organization of highly differentiated post-mitotic cells (e.g.: neurons, myocytes, and hepatocytes) may contribute to cell-specific expression of TTD features. We have shown that in post-mitotic cells, TFIIH is bound to promoters with a much longer residence time than in proliferative cells [46]. A possible explanation for this static behavior is that in these post-mitotic terminally differentiated cells, a large part of the transcription program is dedicated to a particular subset of genes defining cellular specificity and housekeeping functions, without the need to continuously switch to transcribe genes that are needed for proliferation (cell cycle, replication, mitosis, etc.). This reduced plasticity of transcription factors can be (partly) reversed by changing conditions such as sudden high doses of genomic insults [46], allowing adaptation to a changing environment. When, due to the TTD mutations, TFIIH function is affected or instable, transcription adaptation is no longer possible and may make cells less responsive to external and internal cues, which may eventually lead to premature cell death or senescence. We have demonstrated that *Ttda*$^{-/-}$ cells have a low steady state level of TFIIH and accordingly have a lower transcriptional activity. The low TFIIH quantity does not seem to be the sole cause of embryonic lethality, since similar low levels of TFIIH are observed in TTD-A patient cell lines, which are compatible with life. It is, however, possible that transcription is more affected in *Ttda*$^{-/-}$ cells, since TTD-A patients still harbor a partial functional TTDA protein. The notion of reduced transcriptional activity in *Ttda*$^{-/-}$ mouse cells argues that this feature may contribute to embryonic lethality. For instance, during certain stages of embryonic development which require high transcriptional capacity, normal embryogenesis may be compromised. Moreover, it cannot be excluded that completely lacking Ttda affects the transcription of a subset of specific genes as shown in cells with XPD-associated TTD mutations defective in activated-transcription of nuclear receptors [47]. In this scenario, the expression of specific genes, essential for development of the embryo, might be disturbed which in turn hinders proper embryogenesis and finally inducing death *in utero*. In both cases, TTDA function appears to extend beyond the previously suggested main function in NER, as it is also important for both development and viability.

Concluding remarks and future prospective

Complete absence of TTDA in mouse cells has revealed that TTDA has an essential function in NER and is essential for embryonic development. The rather mild TTD-phenotype observed in TTD-A patients is due to the presence of partly functional mutant proteins.

The sensitivity to endogenously produced oxidative DNA lesions in *Ttda*$^{-/-}$ cells suggests that TTDA (and likely the entire TFIIH) has additional functions in DNA repair extending beyond NER, causing synergistic effects when inactivated. Recently, it has also been shown that the ribosome biogenesis and maturation are disturbed in TTD patient ES cells [48]. Interestingly, neurological abnormality is a feature that is found in a number of genetic diseases caused by mutations in ribosomal genes [49]. The lethal phenotype observed in *Ttda*$^{-/-}$ embryos is possibly the result of several defects, such as insufficient levels of TFIIH needed for transcription in highly proliferative tissues, ribosome biogenesis, impairment in the activated transcription of specific genes, and unrepaired lesions endogenously induced by e.g. oxidizing agents.

The problem of endogenously produced DNA lesions is one of the major black holes in our knowledge on DNA repair. The involvement of transcription-coupled repair (TCR) in the process for oxidative DNA damage, similar to TC-NER for bulky lesions, has long been a matter of debate within the DNA repair field. However, recently a very sensitive assay to monitor strand-specific repair of both bulky lesions and oxidative DNA lesions was developed, by combining FISH (using strand-specific gene probes) with a Comet-assay [50]. This novel assay unequivocally proved that oxidative DNA lesions block (or at least transiently inhibit) the transcription machinery and require TC-NER factors to resolve stalled RNA polymerases. It is very important to determine how TTDA (or TFIIH) is implicated in repair of endogenously produced lesions and whether this could explain the phenotype. Most endogenous DNA lesions however do not interfere with the RNA polymerase II elongation *in vitro*, although increasing evidence suggests that BER repair-intermediates do so [50,51].

In order to explain the contribution of endogenously produced DNA lesions with respect to cancer and aging, it is important to understand the non-photosensitive form of TTD of which patients also exhibit developmental and premature aging features. Nothing is currently known about the function of C7orf11/TTDN1, i.e. the gene mutated in a small proportion of non-photosensitive TTD cases that show normal response to UV-light and TFIIH steady state level [13,14]. To investigate which genes are mutated within the non-photosensitive form of TTD could lead to relevant information on the origin of the pathological TTD features in general.

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REFERENCES

[1] O.D. Scharer, Nucleotide excision repair in eukaryotes, in: DNA Repair, Mutagenesis, and Other Responses to DNA Damage, Cold Spring Harb. Perspect. Biol. 5 (2013) 85–103.
[2] L. Schaeffer, R. Roy, S. Humbert, V. Moncollin, W. Vermeulen et al., DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor, Science 260 (1993) 58–63.
[3] E. Compe, J.M. Egly, TFIIH: when transcription met DNA repair, Nat. Rev. Mol. Cell Biol. 13 (2012) 343–354.
[4] K. Sugasawa, J. Akagi, R. Nishi, S. Iwai, F. Hanaoka, Two-step recognition of DNA damage for mammalian nucleotide excision repair: directional binding of the XPC complex and DNA strand scanning, Mol. Cell 36 (2009) 642–653.
[5] J.M. Egly, The 14th Datta Lecture. TFIIH: from transcription to clinic, FEBS Lett. 498 (2001) 124–128.
[6] M. Zurita, C. Merino, The transcriptional complexity of the TFIIH complex, Trends Genet. 19 (2003) 578–584.
[7] A.R. Lehmann, DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy, Biochimie 85 (2003) 1101–1111.
[8] J. de Boer, J.H. Hoeijmakers, Nucleotide excision repair and human syndromes, Carcinogenesis 21 (2000) 453–460.
[9] M. Stefanini, E. Botta, M. Lanzafame, D. Orioli, Trichothiodystrophy: from basic mechanisms to clinical implications, DNA Repair (Amst) 9 (2010) 2–10.
[10] F. Coin, J.C. Marinoni, C. Rodolfo, S. Fribourg, A.M. Pedrini, et al., 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.
[11] J.H. Hoeijmakers, DNA damage, aging, and cancer, N. Engl. J. Med. 361 (2009) 1475–1485.
[12] 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.
[13] E. Botta, J. Offman, T. Nardo, R. Ricotti, G. Zambruno, et al., Mutations in the C7orf11 (TTDN1) gene in six nonphotosensitive trichothiodystrophy patients: no obvious genotype-phenotype relationships, Hum. Mutat. 28 (2007) 92–96.
[14] K. Nakabayashi, D. Amann, Y. Ren, U. Saarialho-Kere, N. Avidan, et al., Identification of C7orf11 (TTDN1) gene mutations and genetic heterogeneity in nonphotosensitive trichothiodystrophy, Am. J. Hum. Genet. 76 (2005) 510–516.
[15] G. Giglia-Mari, F. Coin, J.A. Ranish, D. Hoogstraten, A. Theil, et al., A new, tenth subunit of TFIIH is responsible for the DNA repair syndrome trichothiodystrophy group A, Nat. Genet. 36 (2004) 714–719.
[16] M. Stefanini, P. Lagomarsini, S. Giliani, T. Nardo, E. Botta, et al., Genetic heterogeneity of the excision repair defect associated with trichothiodystrophy, Carcinogenesis 14 (1993) 1101–1105.
[17] W. Vermeulen, A.J. van Vuuren, M. Chipoulet, L. Schaeffer, E. Appeldoorn, et al., Three unusual repair deficiencies associated with transcription factor BTF2(TFIIH): evidence for the existence of a transcription syndrome, Cold Spring Harb. Symp. Quant. Biol. 59 (1994) 317–329.
[18] W. Vermeulen, E. Bergmann, J. Auriol, S. Rademakers, P. Frit, et al., Sublimiting concentration of TFIIH transcription/DNA repair factor causes TTD-A trichothiodystrophy disorder, Nat. Genet. 26 (2000) 307–313.
[19] J.A. Ranish, S. Hahn, Y. Lu, E.C. Yi, X.J. Li, et al., Identification of TFB5, a new component of general transcription and DNA repair factor IIH, Nat. Genet. 36 (2004) 707–713.
[20] M. Vitorino, F. Coin, O. Zlobinskaya, R.A. Atkinson, D. Moras, et al., Solution structure and self-association properties of the p8 TFIIH subunit responsible for trichothiodystrophy, J. Mol. Biol. 368 (2007) 473–480.
[21] D.E. Kainov, L.A. Selth, J.Q. Svejstrup, J.M. Egly, A. Poterzsman, Interacting partners of the Tfb2 subunit from yeast TFIIH, DNA Repair (Amst) 9 (2010) 33–39.
[22] F. Coin, L. Proietti De Santis, T. Nardo, O. Zlobinskaya, M. Stefanini, et al., p8/TTD-A as a repair-specific TFIIH subunit, Mol. Cell 21 (2006) 215–226.
[23] M. Fregoso, J.P. Laine, J. Aguilar-Fuentes, V. Mocquet, E. Reynaud, et al., DNA repair and transcriptional deficiencies caused by mutations in the Drosophila p52 subunit of TFIIH generate developmental defects and chromosome fragility, Mol. Cell. Biol. 27 (2007) 3640–3650.
[24] J. Nonnekens, S. Cabantous, J. Slingerland, P.O. Mari, G. Giglia-Mari, In vivo interactions of TTDA mutant proteins within TFIIH, J. Cell Sci. 126 (2013) 3278–3283.
[25] G. Giglia-Mari, C. Miquel, A.F. Theil, P.O. Mari, D. Hoogstraten, et al., Dynamic interaction of TTDA with TFIIH is stabilized by nucleotide excision repair in living cells, PLoS Biol. 4 (2006) e156.
[26] D. Hoogstraten, A.L. Nigg, H. Heath, L.H. Mullenders, R. van Driel, et al., Rapid switching of TFIIH between RNA polymerase I and II transcription and DNA repair in vivo, Mol. Cell 10 (2002) 1163–1174.
[27] A.F. Theil, J. Nonnekens, N. Wijgers, W. Vermeulen, G. Giglia-Mari, Slowly progressing nucleotide excision repair in trichothiodystrophy group A patient fibroblasts, Mol. Cell. Biol. 31 (2011) 3630–3638.
[28] E. Eveno, F. Bourre, X. Quilliet, O. Chevallier-Lagente, L. Roza et al., Different removal of ultraviolet photoproducts in genetically related xeroderma pigmentosum and trichothiodystrophy diseases, Cancer Res. 55 (1995) 4325–4332.
[29] A.F. Theil, J. Nonnekens, B. Steurer, P.O. Mari, J. de Wit, et al., Disruption of TTDA results in complete nucleotide excision repair deficiency and embryonic lethality, PLoS Genet. 9 (2013) e1003431.
[30] J. de Boer, J. de Wit, H. van Steeg, R.J. Berg, H. Morreau, et al., A mouse model for the basal transcription/DNA repair syndrome trichothiodystrophy, Mol. Cell 1 (1998) 981–990.
[31] Y. Zhou, H. Kou, Z. Wang, Tfb5 interacts with Tfb2 and facilitates nucleotide excision repair in yeast, Nucleic Acids Res. 35 (2007) 861–871.
[32] A. de Vries, C.T. van Oostrom, F.M. Hofhuis, P.M. Dortant, R.J. Berg, et al., Increased susceptibility to ultraviolet-B and carcinogens of mice lacking the DNA excision repair gene XPA, Nature 377 (1995) 169–173.
[33] H. Miyauchi-Hashimoto, K. Tanaka, T. Horio, Enhanced inflammation and immunosuppression by ultraviolet radiation in xeroderma pigmentosum group A (XPA) model mice, J. Invest. Dermatol. 107 (1996) 343–348.
[34] H. de Waard, J. de Wit, T.G. Gorgels, G. van den Aardweg, J.O. Andressoo, et al., Cell type-specific hypersensitivity to oxidative damage in CSB and XPA mice, DNA Repair (Amst) 2 (2003) 13–25.
[35] T. Helleday, E. Petermann, C. Lundin, B. Hodgson, R.A. Sharma, DNA repair pathways as targets for cancer therapy, Nat. Rev. Cancer 8 (2008) 193–204.
[36] H. Lans, J.M. Lindvall, K. Thijssen, A.E. Karambelas, D. Cupac et al., DNA damage leads to progressive replicative decline but

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extends the life span of long-lived mutant animals, Cell Death Differ. 20 (2013) 1709–1718.

[37] J.O. Andressoo, J.H. Hoeijmakers, H. de Waard, Nucleotide excision repair and its connection with cancer and ageing, Adv. Exp. Med. Biol. 570 (2005) 45–83.

[38] I. van der Pluijm, G.A. Garinis, R.M. Brandt, T.G. Gorgels, S.W. Wijnhoven, et al., Impaired genome maintenance suppresses the growth hormone—insulin-like growth factor 1 axis in mice with Cockayne syndrome, PLoS Biol. 5 (2007) e2.

[39] A.H. Sarker, S.E. Tsutakawa, S. Kostek, C. Ng, D.S. Shin et al., Recognition of RNA polymerase II and transcription bubbles by XPG, CSB, and TFIIH: insights for transcription-coupled repair and Cockayne syndrome, Mol. Cell 20 (2005) 187–198.

[40] P.C. Hanawalt, G. Spivak, Transcription-coupled DNA repair: two decades of progress and surprises, Nat. Rev. Mol. Cell Biol. 9 (2008) 958–970.

[41] H. Menoni, J.H. Hoeijmakers, W. Vermeulen, Nucleotide excision repair-initiating proteins bind to oxidative DNA lesions in vivo, J. Cell Biol. 199 (2012) 1037–1046.

[42] P. Schwertman, A. Lagarou, D.H. Dekkers, A. Raams, A.C. van der Hoek, et al., UV-sensitive syndrome protein UVSSA recruits USP7 to regulate transcription-coupled repair, Nat. Genet. 44 (2012) 598–602.

[43] 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.

[44] S. Dubaele, L. Proietti De Santis, R.J. Bienstock, A. Keriel, M. Stefanini, et al., Basal transcription defect discriminates between xeroderma pigmentosum and trichothiodystrophy in XPD patients, Mol. Cell 11 (2003) 1635–1646.

[45] V. Viprakasit, R.J. Gibbons, B.C. Broughton, J.L. Tolmie, D. Brown, et al., Mutations in the general transcription factor TFIIH result in beta-thalassaemia in individuals with trichothiodystrophy, Hum. Mol. Genet. 10 (2001) 2797–2802.

[46] G. Giglia-Mari, A.F. Theil, P.O. Mari, S. Mourgues, J. Nonnekens, et al., Differentiation driven changes in the dynamic organization of Basal transcription initiation, PLoS Biol. 7 (2009) e1000220.

[47] A. Keriel, A. Stary, 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.

[48] J. Nonnekens, J. Perez-Fernandez, A.F. Theil, O. Gadal, C. Bonnart, et al., Mutations in TFIIH causing trichothiodystrophy are responsible for defects in ribosomal RNA production and processing, Hum. Mol. Genet. 22 (2013) 2881–2893.

[49] E.F. Freed, F. Bleichert, L.M. Dutca, S.J. Baserga, When ribosomes go bad: diseases of ribosome biogenesis, Mol. Biosyst. 6 (2010) 481–493.

[50] J. Guo, P.C. Hanawalt, G. Spivak, Comet-FISH with strand-specific probes reveals transcription-coupled repair of 8-oxoGuanine in human cells, Nucleic Acids Res. 41 (2013) 7700–7712.

[51] T. Thorslund, C. von Kobbe, J.A. Harrigan, F.E. Indig, M. Christiansen, et al., Cooperation of the Cockayne syndrome group B protein and poly(ADP-ribose) polymerase 1 in the response to oxidative stress, Mol. Cell. Biol. 25 (2005) 7625–7636.

[52] J.A. Marteijn, H. Lans, W. Vermeulen, J.H.J. Hoeijmakers, Understanding nucleotide excision repair and its roles in cancer and ageing, Nat. Rev. Mol. Cell Biol. 15 (2014) 465–481.

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