



## Splitting hairs – discovery of a new DNA repair and transcription factor for the human disease trichothiodystrophy

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### Abstract

*The gene responsible for the TTD-A group of the DNA repair deficient disease trichothiodystrophy has been identified as a small, 8 kDa, component of the transcription factor TFIIH which contributes to the stability and concentration of TFIIH in vivo.*

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For those who think that DNA repair may have matured to the point that the field has few surprises, think again. Two new studies just published report the discovery of a new repair gene that encodes a component of the transcription factor TFIIH. This component had hitherto been overlooked because of its small size, only 8 kDa, one-tenth the size of the larger repair helicases, XPB and XPD [1,2]. This component is mutated in three patients with the disease trichothiodystrophy (TTD) who had provisionally been classified as TTD-A, but shown previously not to carry mutations in the *XPB* or *XPD* genes, like the majority of TTD patients [2].

TTD is one of the diseases within the category of UV sensitive disorders epitomized by the repair deficient xeroderma pigmentosum (XP), which includes the XP variant (XP-V), Cockayne syndrome (CS), Cranial Oculo Facio Skeletal syndrome (COFS), UV sensitive syndrome (UV<sup>S</sup>) plus some patients who have combined XP and CS symptoms [3]. TTD patients have sulfur-deficient brittle hair with characteristic “tiger tail” banding when examined in polarized light, skin photosensitivity without increased pigmentation or cancer and immune deficiency [3,4]. The initial discovery of XP supported a simple notion that a defect in repair of UV damage resulted in increased mutations that led to the development of skin cancer [5]. Subsequent research into these diseases has strikingly increased our appreciation of the large number

of organ systems that are affected by repair deficiencies and how much more complex are their genetic, molecular and clinical manifestations. The new reports highlight the power of modern genomic and proteomic approaches to the study of macromolecular complexes, the continued value of yeast as a stepping-stone to human studies, and the invaluable contributions made by astute clinical observations of rare individual cases of these genetic diseases.

The study of repair deficient diseases has made important inroads into many fundamental areas of transcription, DNA replication, recombination, cell cycle regulation, and genomic and protein stability. These processes all have in common the functioning of macromolecular complexes that involve many individual proteins. The new reports build on the association between repair and transcription that was made by the discovery that two of the XP gene products, the helicases XPB and XPD, were components of the transcription factor TFIIH which is involved in basal and activated transcription [6,7] (Fig. 1). A related discovery showed that transcribing genes were more rapidly repaired than non-transcribed genes, leading to the division of the nucleotide excision repair (NER) pathway into two branches: global genome repair (GGR) and transcription-coupled repair (TCR) [8]. An increased risk in humans for skin cancer is more consistently associated with the GGR pathway, but the TCR pathway exhibits the more complex variety of

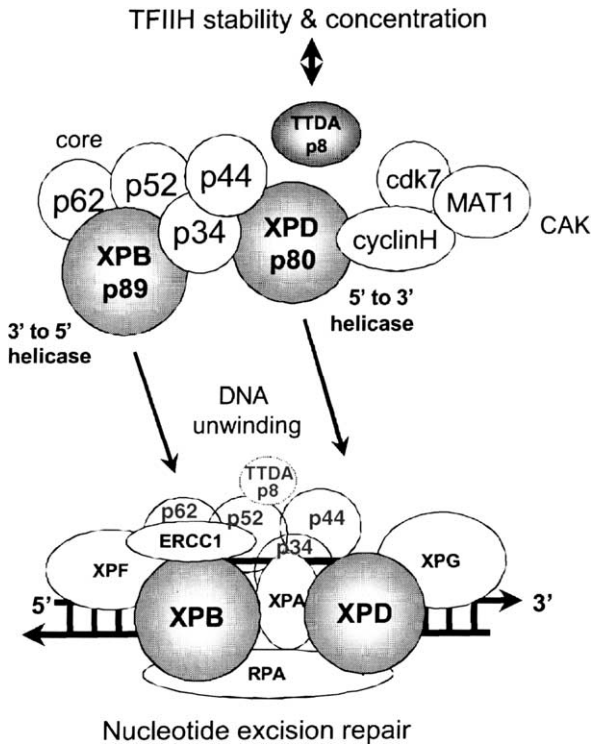


Fig. 1. The nine components of the basal transcription factor TFIIF consist of a core of five proteins, p34, p44, p52, p62, and XPB (p89) that is required for NER, linked through XPD to a group of three proteins, cyclin H, MAT1, and cdk7 (CAK), that is involved in RNA pol II phosphorylation and cell cycle regulation [16]. Each of the three proteins involved in TTD are shown shaded. The tenth component of TFIIF, TTDA, p8, contributes to the stability and concentration of TFIIF in the cell and protection from degradation in vitro. The whole of TFIIF is required for basal and activated transcription. Nucleotide excision repair is shown at the bottom: XPA and RPA represent the verification complex that binds to a damaged site subsequent to damage recognition by the XPGHR23B complex; XPG is the 3'-nuclease; XPF/ERCC1 is the 5'-nuclease. XPB and XPD are involved in unwinding a damaged region of DNA to facilitate endonuclease cleavage and excision of the damaged oligonucleotide, but do not necessarily dissociate from the core during unwinding. The other core components of TFIIF are shown in transparent outline with XPB and XPD on the NER complex.

lapping peptides were found that corresponded to a previously uncharacterized small open reading frame YDR079c-a that was enriched two-fold. This was analyzed in detail and found to represent an unknown small 8 kDa component of the TFIIF transcription factor that was designated TFB5. Deletion of TFB5 caused UV sensitivity, slow growth on a number of carbon sources, reduced basal and activated transcription, and reduced recruitment of TFIIF to promoters. A database search identified a 71 amino acid protein (p8) that was a putative human homolog of TFB5, designated GTF2H5 [2]. Detailed analysis confirmed that this protein played a similar role in human cells to that played by TFB5 in yeast. It participated in UV repair, was localized to sites of UV damage, was involved in maintaining high levels of TFIIF, and most importantly the gene carried inactivating mutations in cells from each of the three TTD-A patients [2]. In human cells unlike yeast the absence of the TTD-A gene product does not seem to affect basal levels of transcription, but mutations in all three TTD-related genes affect levels of intracellular TFIIF [9].

The mechanistic details of how mutations in XPB, XPD and now GTF2H5 each can give rise to the TTD phenotype remain enigmatic. One view is that some classes of cells such as those that are terminally differentiated place a special demand on the transcription of specialised genes. Low levels of TFIIF could then prematurely impair these and other functions in hair, brain and other tissues, such as has been shown for beta-globin in TTD-associated thalassemias [10]. One intriguing notion is that since GTF2H5 is required for stability and concentration of TFIIF in vivo, could it be involved as a cofactor for a ubiquitylation system for TFIIF? Ubiquitylation has become an important mechanism for regulating the response of cells to UV damage and has been associated with transcription-coupled and global genome repair [11] and bypass replication [12]. The XPB component of TFIIF also interacts with a component of the 26S proteasome [13]. It would not be inconceivable to find a similar mechanism associated with the contribution that the new factor GTF2H5 makes to TFIIF stability.

These studies highlight the greatly increased technical capacity now available in yeast genetics, proteomics and genomics. But they also enhance the value of clinical observations and of reporting rare patients. How many other uncharacterized patients remain to be identified with repair genes? A recent genome-wide study using yeast deletion mutants has identified a large number of genes, in the hundreds, that can contribute to resistance to UV damage [14], many of which do not have self-evident properties related to damage response, and have not been evaluated in humans for roles in UV response. We may be missing human patients because mutations in certain genes would be so critical that the patients rarely survive, as is the case for the UV repair 5'-endonuclease cofactor ERCC1. Or their phenotypes may be so mild, such as in XPE [3] and UV<sup>S</sup> patients [15], as to blend with the population of sun-sensitive individuals that have type I skin.

symptoms, involving developmental and neurodegenerative disorders, that are exhibited in XP, CS, TTD and COFS patients. Since a number of studies had shown that TTD-A patients had low levels of TFIIF [9], it was logical to search among the other TFIIF components for candidate mutations but none were found [2], even though in yeast each of the core components leads to a UV sensitive phenotype when mutated.

Ranish et al. [1] isolated yeast RNA pol II preinitiation complexes by differential labeling of proteins that would bind to initiation sequences in the presence of TATA-binding protein (TBP), but not in its absence. These proteins were then further purified, digested to peptides and analyzed by mass spectrometry. Peptides that were enriched by the presence of TBP in these extracts corresponded to many known components of the RNA pol II transcription machinery. Two over-

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