TFIIH: a key component in multiple DNA transactions
Jan HJ Hoeijmakers*, Jean-Marc Egly† and Wim Vermeulen*

The transcription factor TFIIH is a versatile, multi-functional protein complex with multiple engagements. Apart from its role in basal transcription, TFIIH is intimately implicated in DNA repair and (probably) in cell cycle control (both of which are required to prevent carcinogenesis) as well as having possible roles in other processes. Thus, it is a striking example of the efficient use of one component for many purposes. Ingeniously, the incorporation of this essential factor into important, but non-essential, mechanisms, such as DNA repair, protects against cancer. The critical role of TFIIH in transcription function renders inactivating TFIIH mutations lethal to cells. Without this transcription connection, such mutations would lead to genetic instability and oncogenesis.

Introduction

One of the transcription factors required for all structural genes is TFIIH. In recent years most of the nine, or more, TFIIH subunits have been either cloned or rediscovered. This work has disclosed unexpected links with other processes and has indicated a strong conservation of the entire complex from yeast to man (Table 1). Several functions have been inferred or demonstrated for individual TFIIH components (Table 1). The complex is equipped with two helicases with opposite polarity, XPB(ERCC3) and XPD(ERCC2), which were originally referred to as DNA repair factors [1-6]. In addition, at least three other TFIIH subunits are directly involved in repair: the yeast (in this review Sadanmyccs) and human subunit TTDA [PO] and a large RNA polymerase holoenzyme, containing most of the factors in a preassembled form [25,26,27**]. Such a 'transcription machine' may have escaped previous detection because of its labile structure. Although the functional status of these super-complexes has yet to be verified, the existence of highly organized DNA-metabolizing 'machineries' such as this [28] is conceptually appealing.

<table>
<thead>
<tr>
<th>Properties of cloned TFIIH subunits.</th>
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<tr>
<td>Human TFIIH cloned gene</td>
</tr>
<tr>
<td>XPB/ERCC3</td>
</tr>
<tr>
<td>XPD/ERCC2</td>
</tr>
<tr>
<td>p52</td>
</tr>
<tr>
<td>p52</td>
</tr>
<tr>
<td>p44</td>
</tr>
<tr>
<td>Mo15/CDK7</td>
</tr>
<tr>
<td>p34</td>
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<tr>
<td>cyclinH</td>
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<tr>
<td>MAT1</td>
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* No known function; †yeast cognate identified in database. See [12**,13**].
All components are already on 'standby', circumventing rate-limiting diffusion of numerous factors.

To date, TFIIH is the only known basal transcription factor with enzymatic activities [10,22]. The presence of the bidirectional helicases, XPB and XPD, implicate TFIIH as a 'helix opener' involved in the generation of an open initiation complex (Fig. 1). This may enable RNA polymerase to load onto the template-strand and promote formation of the first phosphodiester bonds. Such a model satisfies the need for ATP hydrolysis in initiation and is consistent with in vitro studies in which the requirement for TFIIH is alleviated when template melting is favoured [29,30*].

A subsequent step in the initiation reaction that may permit 'promoter clearance' [31] is the CTD phosphorylation of RNA polymerase II. The CTD is composed of multiple heptad repeat units. Genetic and biochemical studies [32,33*,34] have identified that CTD acts as a target for interaction with activator proteins at the promoter. Its phosphorylation may disrupt this association and release the polymerase to allow elongation to proceed [35]. Although the in vitro transcription for some promoters does not depend on CTD phosphorylation [36], the CTD is essential in vivo [11]. The Cdk7/Cyclin H/Mat1 trimer within the context of TFIIH is implicated in at least part of the CTD phosphorylation [37*,38]. Available evidence suggests that TFIIH is not involved in elongation itself [39], but because of the difficulty of isolating bona fide elongation complexes this issue has not been conclusively solved.

**TFIIH, DNA repair and human disorders**

The nucleotide excision repair (NER) pathway eliminates a wide variety of DNA lesions, including UV-induced pyrimidine dimers and numerous chemical adducts [40]. This mechanism is also highly conserved from yeast to man [41]. The core reaction has recently been reconstituted in vitro using purified components [42*,43*]. From the known or presumed functions of the identified proteins the contours of a reaction mechanism emerge [41,44]. The XPA protein involved in damage recognition recruits TFIIH, RPA and the two structure-specific endonucleases, XPG and the ERCC1 complex, which cleave the damaged strand five bases downstream and ~22 bases upstream of the injury respectively [45,46]. After peeling off the lesion-containing 2-7-29-mer, the resulting single strand gap is filled by DNA repair synthesis and ligation (for a review, see [41]). Evidence has been presented that a fraction of most yeast NER factors resides in a large 'repairosome' [47]. Thus, as with transcription, a preassembled 'repair machine' may exist. Obviously, DNA damage poses logistical problems for transcription and replication. To avoid a long interruption of transcription mediated by slowly removed lesions (e.g. pyrimidine dimers) [48], a specialized NER subpathway accomplishes the preferential elimination of such damage from the transcribed strand of active genes [49]. This transcription-coupled repair process involves the CSA and CSB products [50,51].

The precise role of TFIIH in NER has not yet been elucidated. Even so, the notion, that both NER endonucleases require transition from a double strand to a single strand region (a splayed-arm structure) for incision [45,46], implies that the DNA at the site of incision is melted. This leaves a niche for the 'helix opener' function of TFIIH (Figure 1). This model explains the ATP requirement of NER and is consistent with the fact that both TFIIH helicases are needed in the pre-incision stage. As in transcription, the 3'→5' unwinding activity of XPB seems more important than the 5'→3' helicase of XPD: a complete inactivation of the XPD helicase function still allows partial repair and is not essential for transcription (G Weeda and W Vermeulen unpublished data). Thus, the physical integrity of XPD may be more important for TFIIH assembly than its functional integrity. In contrast, inactivation of the XPB helicase function completely abrogates repair and transcription, even in a semi-dominant fashion [52]. No clear role for the kinase activity of TFIIH in NER has been documented; however, serine/threonine phosphatase inhibitor studies suggest that protein phosphorylation is implicated in modulating the pre-incision stage (R Wood, personal communication). It is unknown whether the TFIIH composition is identical in transcription and NER.

NER deficiencies are known to underlie the rare, genetic condition, xeroderma pigmentosum (XP). This prototype repair syndrome is manifested predominantly as cutaneous symptoms: sun-sensitivity, pigmentation abnormalities and a >2000-fold increased risk of skin cancer in sun-exposed parts of the body (Table 2), reflecting the role of NER in counteracting UV genotoxicity. Seven complementation groups (XP-A to XP-G), representing a similar number of genes, have been identified [41,53]. Inactivation of CSA and CSB, involved in transcription-coupled repair, result in two complementation groups of Cockayne syndrome (CS). This multi-systemic disease shares the symptom of photosensitivity with XP, and also exhibits severe developmental and mental impairment, including growth retardation and neurodysmyelination, but no skin cancer (Table 2) [53,54]. As it is known that individuals lacking XPA are totally defective in NER, including transcription-coupled repair, but fail to exhibit typical CS symptoms, it is probable that the CS products may have an additional non-repair function. The clinical spectrum associated with defects in TFIIH subunits is even more complex. Some TFIIH patients display only XP symptoms, some have combined XP/CS symptoms and others display the photosensitive form of trichothiodystrophy (TTD) [59*]. The last disorder shares many symptoms with CS, but the definitive symptom is brittle hair and nails, due to a vastly reduced content of
cysteine-rich matrix proteins in these structures (Table 2; for a review, see [55]). Mutations in at least three TFIIH genes are implicated in TTD: XPD (for the majority of patients), XPF and TTDA (only one recorded patient) [41]. Analysis of XPD in XP, XP/CS and TTD patients [56–58] is consistent with the idea that each syndrome is caused by different mutations in this gene. In view of the dual functionality of TFIIH, it is conceivable that a mutation can affect either one or both of the functions of XPD (Table 3) [9,41].

Impairment of only the NER function readily explains the classic XP features. Inactivation of the transcription function is obviously lethal as confirmed by gene targeting in mice (G Weeda, unpublished data). Even so, a slight impairment of transcription function could affect only a limited set of genes, which critically demand optimal TFIIH functioning [9,41]. It could be, for instance, that neurodysmyelination in CS and TTD results from a crippled TFIIH, affecting expression of a gene involved in myelination. One can envisage that a strong secondary structure in a promoter requires the full unwinding capacity (i.e. a maximally fit complex), to permit efficient transcription. This combined ‘repair/transcription syndrome’ concept dissects the pleiotropic clinical features into those derived from a NER defect and those explained by a subtle transcriptional impairment. It provides a rationale for non-photosensitive (NER+) TTD (and CS)
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Table 2

Summary of main clinical symptoms of TFIIH-related disorders.

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>XP</th>
<th>XP/CS</th>
<th>CS</th>
<th>TTD</th>
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<tbody>
<tr>
<td>Sun-sensitivity</td>
<td>+</td>
<td>++</td>
<td>+*</td>
<td>+*</td>
</tr>
<tr>
<td>Abnormal pigmentation</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Skin cancer</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Neurosyphilinization</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bird-like facies</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth defect</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypogonadism</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Brittle hair and nails</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Scaling of skin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* TTD and CS can patients occur without sun-sensitivity and NER defect.

When the CS features in TTD are due to a basal transcriptional problem, this should also apply to the other complementation groups with CS patients (i.e. CS-A, CS-B and XP-G). Although the CSA, CSB (and probably XPG) proteins are not vital and, therefore, not essential for transcription, they may influence TFIIH functioning indirectly (Table 3). For instance, CSA and CSB could have an auxiliary function for TFIIH in transcription and in transcription-coupled (but not global genome) repair.

Table 3

For further explanation see text. For clinical features see Table 2. Symbol designation: + normal function/stability, +/- partly affected function/stability, - severely impaired function. TFIIH transcription function may be indirectly affected by mutations in XPD, CSA and CSB. In addition, XPB affects total NER, CSS affects only transcription-coupled repair, only the NER subpathway of transcription-coupled repair is disturbed; Recently several CS patients without a NER defect were identified (A Raams and NGJ Jaspera, unpublished data). There may be disorders related to non-photosensitive variants of TTD and CS such as Netherton syndrome, Brain-Hair syndrome, Pollitt syndrome, and similar diseases [9].
shown to be able to phosphorylate cell cycle CDKs [20] and its involvement in cell-cycle control is supported by genetic evidence in *S. cerevisiae* [66*], thus, TFIIH may have links with cell cycle regulation as well.

Approximately 50% of the CAK sub-complexes in a cell are in a free form. Both the free and TFIIH-associated CAK (sub)complexes are able to activate other CDKs, but only the TFIIH–CAK complex is capable of CTD phosphorylation [15] (CAK must be activated by phosphorylation too). Within the set of CTD-bound proteins, another CDK/cyclin pair has been discovered in yeast, designated SRB10 and SRB11 respectively [33*]. It is possible that CAK is a target of this kinase or vice versa or that they activate each other. Alternatively, they may work independently on the CTD and/or on CDKs. In contrast with the cell-cycle CDK/cyclin pairs, no cell cycle regulation of CAK has been observed [20]. Thus, if CAK has a role cell cycle control this should be consistent with a non-cell cycle-dependent expression profile.

**Is TFIIH involved in other processes?**

If one considers the multiple roles of TFIIH that have been addressed in this review, it is conceivable that this factor may be involved in other, as yet, unidentified processes. Epistatic analysis of NER mutants of TFIIH in yeast has failed to reveal an involvement of the complex in the two other major multistep repair pathways: recombination repair (represented by the *RAD52* epistasis group), and post-replication repair, defined by the *RAD6* group of mutants [40]. A caveat in these studies, which even applies to conditional mutants, is the fact that only a limited set of mutations in TFIIH subunits is viable. Even so, hints for additional relationships come from some rare *rad3* and *rad25* yeast alleles (encoding the XPD and XPB helicase subunits of TFIIH, respectively) which have increased rates of specific mutations and of mitotic recombination independent of the NER deficiency [67,68]. The available evidence suggests that Rad3 (and, thus, probably also Rad25, XPD and XPB) are involved in the stability of double strand breaks, suppressing homologous recombination [69*].

Interestingly, two other NER factors, Rad1 and Rad10, comprising the yeast 5′ incision complex, also have a dual functionality in a mitotic recombination pathway [70]. Thus, it is possible that TFIIH, together with the NER (ERCC1) 5′-incision complex, also has a role in recombination.

**Conclusions and perspectives**

What might be the common denominator for TFIIH in repair, transcription and cell cycle control? One of the first processes registering DNA damage must be transcription that continuously subjects large, vital parts of the genome to a rigorous test for integrity. DNA damage triggers a cascade of events. First, overall transcription, even of undamaged genes, being temporarily reduced (C Backendorf, unpublished data). This presumably avoids cellular homeostasis imbalance, otherwise selectivity large genes would suffer. Second, a cell cycle pause is induced, involving p53 and numerous cell cycle checkpoint genes. Third, DNA repair has to be activated. TFIIH has all the connections and components to modulate these responses.

The following scenario can therefore be envisaged. When RNA polymerase stumbles upon a lesion, TFIIH is recruited from the transcription into the NER mode enabling repair and simultaneously shutting down transcription. TFIIH CAK activity may trigger cell-cycle arrest by its ability to regulate CDK activities. The mechanism controlling cell-cycle progression must be operational throughout the cell cycle, which fits with the constitutive CAK expression that is observed. When DNA damage is so severe that it entails a risk of oncogenesis, the apoptotic suicide mechanism is triggered, involving further accumulation of the p53 ‘master regulator’. Local high concentrations of p53, in turn, may inhibit TFIIH, thereby blocking repair and facilitating apoptosis. The reports of interactions between TFIIH subunits and p53 are consistent with this idea [71].

The versatile application of TFIIH may be best realized when the transcription, repair and cell-cycle machineries are intimately connected. Perhaps such a connection can be efficiently accomplished in well organized large DNA-metabolizing machineries, which may be represented by the multi-purpose TFIIH complex.

**Acknowledgements**

We gratefully acknowledge the stimulating discussions with all members of our laboratories as well as our colleagues. Our research is supported by the Dutch Scientific Organizations NWO and SON, the Dutch Cancer Society, Human Frontiers and the Louis Jeantet Foundation. Finally, we sincerely apologise for the restricted number of references and the emphasis on recent literature that is required for the format of this review, resulting in undercitations of older literature and frequent reference to reviews.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- **of special interest**
- **of outstanding interest**


The core components of yeast TRIH, Tfb1 and Ssl1, are shown to be directly engaged in NER Analysis of temperature-sensitive repair deficient mutants

8. Wano 2, Buratowski S, Svejstrup JQ, Feaver WJ, Wu X, Kombera complex stability is more important for the transcription function but not the NER function, is sensitive to temperature shift, but not the

0.

TfllH subunits and the NER complementation groups which display ex-

clinical impact, because many other unexplained syndromes could also fall of CDK-activating kinase and RNA polymerase II. Cell 1005,73:73-77.

The yeast holo-TFIIH can be separated into core TFIIH and two sub-complexes, which, encode subunits of transcription factor IIAH, are required for nucleotide excision repair and RNA polymerase II transcription. Mol Cell Biol 1995, 15:2288-2293.

The core components of yeast TFIIH, Tfb1 and Ssl1, are shown to be directly engaged in NER. Analysis of temperature-sensitive repair-deficient mutants for in vitro NER and transcription initiation, reveal that the transcription function, but not the NER function, is sensitive to thermal shift, but not the NER function. This dissects the dual functionality of TFIIH and suggests that complex stability is more important for the transcription function than for the repair function. The significant proportion of temperature-sensitive mutants in yeast TFIIH may have important clinical implications for human patients with mutations in the same genes.


In vitro and in vivo experiments support the idea that the entire TFIIH core is involved in NER. A specific association is found between mutations in TFIIH subunits and the NER complementation groups which display extreme clinical heterogeneity, including the pleiotropic IC57 features. Evidence is provided that MO15 plays a role in transcription and repair both in vivo and in vitro.


Yeast holo-TFIIH can be separated into core TFIIH and a sub-complex called TFIIK. The latter, comprising three polypeptides, is able to phosphorylate the CTD of RNA polymerase II. The 33 kDa subunit of TFIIK was identified as p34cdk7/cdc20 kinase family, implicated in cell cycle control. In human MO15 (CDK7), the catalytic subunit of cdk activating kinase (CAK).


The intrinsic CTD kinase activity of the transcription/repair factor TFIIH in humans is shown to be due to TFIIH associated MO15. Either MO15 or Cdk7 is the Cdc2-related catalytic subunit of CAK. The CTD kinase activity can be separated from TFIIH. Two other polypeptides co-purify with MO15, one of which is identified as cyclin H. Evidence is provided that MO15 plays a role in transcription and repair both in vivo and in vitro.


27. Osajpow V, Tassan JP, Nigg EA, Schibler U: A mammalian


Following, and in accordance with, previous observations in yeast (S. cerevisiae) [24], the isolation of a mammalian RNA polymerase holoenzyme is reported in this paper. Using anti-MO15 monoclonal antibodies, co-immunoprecipitation of all basal initiation factors from rat liver extracts is demonstrated. This immunoprecipitate is sufficient to induce transcription in vitro, but does not contain liver-specific transcriptional activators.


30. Holstege FCV, Van der Vliet PC, Timmore MTH: Opening of an RNA polymerase II promoter occurs in two distinct steps and requires the basal transcription factors II E and IIH. EMBO J 1996, in press.

Heteroduplex DNA around the transcriptional start site alleviates the require-

ment of TFIIH and TFIIJ. With a chemical detection method for single strand DNA (KMnO4) it is shown that promoter opening depends on the complete preinitiation complex (DBpolFEH) and occurs in two discrete steps, one of which requires ATP hydrolysis. These findings support a model for the creation of a single-strand region generated by the TFIIH-associated DNA helicases.


Two of the transcriptional regulatory proteins (Srb5b) in the yeast RNA pol-

ymerase II holoenzyme are identified as a kinase-cyclin pair. Holoenzymes lacking this kinase pair are deficient in CTD phosphorylation and transcriptional responses to galactose induction in vivo, whereas transcription in vitro is not affected.

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38. With the aid of a thermoventive mutant of Kin28, it is shown that Kin28 is required for efficient transcription in vivo. However, CAK activity, both in vitro and in vivo, does not depend on active Kin28. This is in contrast to its human homolog CDK7, which was shown to be the catalytic subunit of CKI. In accordance with the mammalian TRIM-associated kinase, however, Kin28 is able to phosphorylate the CTD of RNA polymerase II.


41. The reconstitution of intron 7, of the core components of the mammalian NER reaction, using recombinant and purified NER factors, is described. The nuclear process is dissected into two stages: damage removal and gap-filling. The current focus for most of the factors and/or NER-factors, including TFIIH, within the lesion elimination step is established.


43. The reconstitution, in vitro, of the core components of the mammalian NER reaction, using recombinant and purified NER factors, is described. The nuclear process is dissected into two stages: damage removal and gap-filling. The crucial role for most of the factors and/or NER-factors, including TFIIH, within the lesion elimination step is established.


45. Describes the reconstitution in vitro nuclear stage of mammalian NER.


66. A photosensitiv TS patient, belonging to XP group D, is described. This patient exhibits the remarkable feature of intermittent loss of the scalp hair during infectious periods. Within a period of a few months, the scalp hair return. This peculiar phenomena of hair loss after fever, may be indicative of a thermoventive mutation in the XPB subunit of TFIIH.


69. In a search for co-essential, using a thermoventive kin28 mutant, these authors uncover genetic interactions with genes implicated in transcription and cell cycle control. The transcription connection is confirmed at the biochemical level with the aid of the temperature-sensitive mutants, showing a concomitant reduction of both transcription and CTD phosphorylation at the restrictive temperature.


A temperature-sensitive mutant of RAD3 is isolated, that has an elevated frequency of recombination between DNA stretches with short sequence homology. Together with previously isolated rad3 alleles, which have a recombination phenotype, it suggests that Rad3 plays an additional role in one of the recombination pathways. In addition to rad3 mutants with a NER-phenotype and/or transcription phenotype, these recombination mutants extend the pleiotropic phenotypical expression of this TFIIH subunit.
