TFIIH: a key component in multiple DNA transactions

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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.

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Abbreviations

CAK	CDK-activating kinase
CDK	Cyclin dependent kinase
CS	Cockayne syndrome
NER	nucleotide excision repair
TTD	trichothiodystrophy
XP	xeroderma pigmentosum

Introduction

One of the basal 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 *Saccharomyces cerevisiae*) proteins Ssl1 and Tfb1 [7•,8••] and one, as yet unidentified, human subunit TTDA [9••]. TFIIH also exhibits a kinase activity for the carboxy-terminal domain (CTD) of the large subunit of RNA polymerase II [10,11] due to Cdk7 (alias MO15), a known cyclin-dependent kinase (CDK) activating kinase [12••,13••,14,15]. Together with cyclin H and Mat1 (also named p36), it forms a trimeric sub-complex [16–18], that is capable of activating other cell cycle controlling CDK/cyclin complexes [19,20]. Thus, a likely link between TFIIH and cell cycle regulation exists. In addition, both p34, which harbours a Ssl1-like zinc-finger, and p52 have been cloned [JM Egly, unpublished data; [21]). Because of the difficulty of isolating the intricate TFIIH complex intact, it is unknown whether some subunits are lost during purification and thus remain unidentified.

Table 1

Properties of cloned TFIIH subunits.

Human TFIIH cloned gene	Yeast TFIIH cloned gene	Protein properties
XPB/ERCC3	*SSL2/RAD25*	3′→5′helicase
XPD/ERCC2	*RAD3*	5′→3′helicase
p62	*TFB1*	?*
p52	*TFB2*	WD-repeats
p44	*SSL1*	2 types of Zn^{2+}-fingers
Mo15/CDK7	*KIN28*	CDK-like kinase
p34	*Scp34*†	Ss11-like Zn^{2+}-finger
cyclinH	*CCL1*	homology to cyclins
MAT1	*TFB3*	ring Zn^{2+}-finger

* No known function; †yeast cognate identified in database. See [12••,13••].

TFIIH in basal transcription

In vitro analyses have supported a model for initiation of basal transcription, involving an ordered assembly of transcription factors at the site of a promoter. This scenario begins with TATA-box binding by TFIID, followed by TFIIB and RNA polymerase II–TFIIF and is completed with the association of TFIIE and TFIIH [10,22]. Understandably, these factors (totalling >40 polypeptides) are operationally defined as separately purified components. *In vitro* systems have the major advantage, but at the same time the intrinsic limitation, that they simplify the complex *in vivo* state. The dynamic chromatin conformation, which poses extra levels of intricacy [23], is not found *in vitro*. Moreover, the DNA and protein concentrations *in vivo* are 100 mg ml^{-1} and 250 mg ml^{-1} respectively (see [24]) that is, beyond the point of crystallization and orders higher than what is found *in vitro*. This dearth of genetic and proteinous material dramatically influences reaction kinetics, complex formation etc. More recently, evidence has emerged for 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.

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 heptapeptide repeats. 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

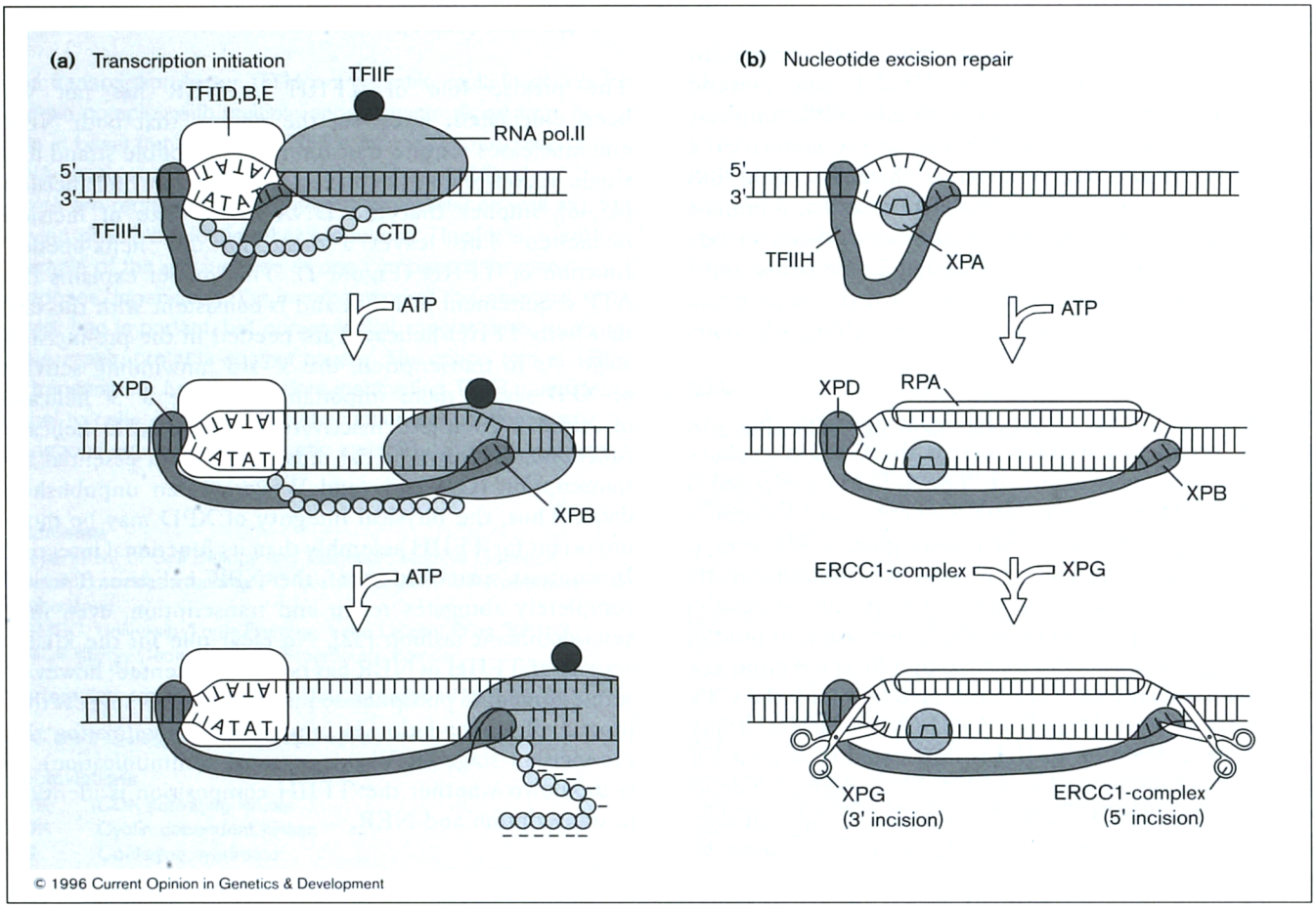
The nucleotide excision repair (NER) pathway eliminates a wide variety of DNA lesions, including UV-induced pyrimidine dimers and numerous chemical adducts [40]. This mechanism is also highly conserved from yeast to man [41]. The core reaction has recently been reconstituted *in vitro* using purified 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) [9••]. The last disorder shares many symptoms with CS, but the definitive symptom is brittle hair and nails, due to a vastly reduced content of

Figure 1



Model for the role of TFIIH in transcription and repair. **(a)** TFIIH in initiation of basal transcription of RNA polymerase II. After binding of the TFIID complex to the TATA box and recruitment of TFIIB and RNA polymerase II together with TFIIF, the assembly of the initiation complex is completed by the association of TFIIE and TFIIH. Several proteins at the site of the promoter interact with the non-phosphorylated carboxy-terminal domain (CTD) of the large subunit of RNA polymerase II. The XPB–XPD bidirectional helicase duo of TFIIH is thought to locally open the DNA helix for loading of the RNA polymerase onto the transcribed strand and to permit synthesis of the first phosphodiester bonds. The CAK sub-complex of TFIIH phosphorylates the CTD, thereby disrupting the interaction of the RNA polymerase with promoter-bound proteins. Allowing promoter clearance and transition into elongation. **(b)** TFIIH in the core of the NER reaction. Lesion recognition presumably involving XPA is followed by the association of ss-DNA binding RPA complex and TFIIH. The 'helix-opener' function accomplished by the XPB and XPD helicases generates a locally melted region. The transition points from duplex to ss-DNA constitute substrates for the structure-specific endonuclease action of the ERCC1-complex and XPG which are responsible for incising the damaged strand approximately 22 bases upstream and 5 bases downstream of the lesion respectively. Since the helicase function of XPB appears more important for NER and transcription than that of XPD we favour XPB unwinding the largest part of the opened regions in transcription and repair.

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), *XPB* 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 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)

Table 2

Summary of main clinical symptoms of TFIIH-related disorders.

Clinical symptoms	XP	XP/CS	CS	TTD
Sun-sensitivity	++	++	+*	+*
Abnormal pigmentation	++	+	–	–
Skin cancer	++	+	–	–
Neurodysmyelination	–	+	+	+
Bird-like facies	–	+	+	+
Growth defect	+/–	+	+	+
Hypogonadism	–	+	+	+
Brittle hair and nails	–	–	–	+
Scaling of skin	–	–	–	+

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

patients, in which only the transcription function of TFIIH might be affected. At the same time, it accounts for the extreme rarity of these syndromes, in general, and the relative abundance of XPD, since this gene tolerates even helicase-inactivating mutations (see above).

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. The clinical picture of some XP-G (XP/CS) patients are more severe than XP-A patients (NGJ Jaspers, A Raams, unpublished data; [59]), although the latter are more defective in NER, suggesting that XPG has an additional non-repair function. Consistent with these predictions are the reports of interactions between the CS proteins and TFIIH components [4,50,60].

How can we rationalize the differences in symptoms between CS and TTD, such as the additional presence of brittle hair and nails in TTD? A significant proportion of TFIIH mutations in yeast yield a temperature-sensitive phenotype [8••,61–63], probably caused by complex instability. It is feasible that these types of mutation also occur among TFIIH patients. Interestingly, two exceptional TTD individuals exhibited a reversible sudden and dramatic worsening of hair brittleness formed during an episode of fever [64•,65], which is in accord with a human, temperature-sensitive TFIIH mutation. Furthermore, these phenomenon suggest it is possible that transcription of the genes for cysteine-rich matrix proteins of hair and nails is affected by TFIIH instability. In other cells, the steady-state levels of TFIIH may be sufficient because *de novo* synthesis is high enough. The cysteine-rich matrix proteins are, however, one of the last gene products produced in very large quantities in keratinocytes before they die. Thus, the hallmark of TTD may be due to a TFIIH-stability problem becoming overt in terminally differentiated cells that are exhausted for TFIIH before completion of their differentiation program. The non-repair CS features can be explained by a malfunctioning of TFIIH, without concomitant complex instability [Table 3]. Future research using CS and TTD mouse models should reveal whether these concepts are realistic.

TFIIH and the cell cycle

Passage through the mammalian cell cycle is controlled by a strict order of successive waves of activation and inactivation of five or more CDKs. One of these activating steps is the CDK phosphorylation of the threonine residue at position 161 within the T-loop, that controls access to the catalytic domain. As mentioned above, the TFIIH CTD kinase is the CDK-activating kinase (CAK), comprising the catalytic subunit Cdk7 [12••,13••], its regulator cyclin H and a Ring-type Zn^{2+}- finger assembly factor, p36 or Mat1 [16–18]. The CAK trimer has been

Table 3

Model for the relation between TFIIH defects and clinical features*.

TFIIH-related disorder†	NER	TFIIH function†† transcription	stability	Documented gene mutation
XP	–	+	+	*XPD*
XP/CS	–	+/–	+	*XPD, XPB, XPG*§
CS (photosensitive)	+/–#	+/–	+	*CSA, CSB*§
CS (non-photosensitive)**	+	+/–	+	?
TTD (photosensitive)	–	+/–	+/–	*XPD, XPB, TTDA*
TTD (non-photosensitive)**	+	+/–	+/–	?

* 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 *XPG*, *CSA* and *CSB*. In addition, *XPG* affects total *NER*, *CSA* and *CSB* 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 Jaspers, 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 selectively 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 sever 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.

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- •• of outstanding interest

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