

Transcription factor IIIH – the protein complex with multiple functions

Minireview

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Transcription factor IIIH (TFIIH) is eukaryotic multi protein complex identified in early 90's. Subsequent years have shown exceptional conservation of its structure from yeast to human. Although initially considered to be exclusively a basal transcription factor responsible for initiation of transcription and transition from initiation to elongation, TFIIH is also important in nucleotide excision repair for opening DNA at the site of a lesion and for recruitment of additional repair factors. Recently it was suggested that intact holocomplex of TFIIH is required for cell cycle regulation. Moreover, mutations in TFIIH subunits lead to three distinct genetic disorders: xeroderma pigmentosum (DNA repair disorder/cancer syndrome), Cockayne syndrome (DNA repair disorder/transcription syndrome/segmental progeria) and trichothiodystrophy (DNA repair disorder/transcription syndrome). This review is focused on the TFIIH structure, its role in transcription, DNA repair and cell cycle regulation and association with some human hereditary disorders.

Keywords: TFIIH, transcription, nucleotide excision repair, cell cycle, human hereditary disorders

TFIIH structure. TFIIH is multifunctional complex of proteins which consists of two sub-complexes: core complex and cyclin-dependent kinase (CDK) - activating kinase (CAK) complex. Core complex consists of seven subunits: xeroderma pigmentosum B (XPB; p89), xeroderma pigmentosum D (XPD; p80), p62, p52, p44, p34, and trichothiodystrophy A (TTD-A; p8) [1, 2]. CAK is composed of three subunits: CDK7 (p40), cyclin H (p34) and menage á trois 1 (MAT1; p32) [3]. XPB and XPD are ATP-dependent DNA helicases. XPB is the biggest subunit of TFIIH, contains seven helicase motifs and belongs to helicase superfamily 2 (SF2) [4]. XPD structure has a RecA-like fold found in the SF1 and SF2 family of helicases, with two domains (HD1 and HD2) [5]. Proteins p62, p52, p44 and p34 were previously called "structural" subunits of TFIIH. Recent studies, however, revealed new functions of some of these subunits. Protein p52 upregulates the ATPase activity of XPB through a direct XPB/p52 contact and anchors XPB to the TFIIH [6, 7]. Protein p44 regulates XPD through direct p44/XPD contact [8] and has ubiquitin ligase activity. Protein p62 interacts with thyroid hormone receptor TR β . This suggests an alternative pathway for nuclear receptor

communication with the general transcription machinery [9]. The last discovered protein TTDA (p8) of the core TFIIH sub-complex carries the name of trichothiodystrophy (TTD) disease. Mutation in this protein cause particular form of TTD termed TTD-A [1].

CDK7 is the biggest subunit of CAK. CDK7 is bifunctional in metazoans: it phosphorylates CDKs participating in the cell-cycle, and is a component of the TFIIH, which phosphorylates the C-terminal domain (CTD) of the largest subunit of RNA polymerase II [10]. MAT1 is involved in CAK anchoring to the core TFIIH in a complex interaction in which both XPD and XPB helicases take part. MAT1 interacts with the CDK7-cyclin H complex and stimulates the CDK7 kinase activity [11].

TFIIH is organized into a ring-like core structure from which globular CAK sub-complex protrudes out [12, 13]. XPD seems to bridge the core and the CAK and allows CAK anchoring to TFIIH [14]. TFIIH composition must be dynamic to adapt its engagement in various cellular processes. The CAK is released from the core during nucleotide excision repair (NER). Recent study shows that xeroderma pigmentosum A (XPA) catalyzes this detachment, which is accompanied

by arriving of the other NER factors. The release of the CAK from the core TFIIH promotes the incision/excision of the damaged oligonucleotide and thereby the repair of the DNA. After completion of DNA repair the CAK reattach to the TFIIH, and transcription is renewed [15].

TFIIH and transcription. Eukaryotic transcription by RNA polymerase II (Pol II) is highly regulated and coordinated action. The first stage of this process, initiation of transcription, requires additional five factors known as “general transcription factors”: TFIIB, TFIID, TFIIE, TFIIF and TFIIH [16]. Altogether, more than 30 polypeptides are needed to form pre-initiation complex on the promoter. The TATA box, located approximately 30 bases upstream of the transcription initiation site (TIS), is specifically recognized by the transcription factor TFIID, forming DNA bend in the site of connection between its amino acid residues and the DNA bases [17]. Structure is further stabilized by the binding of TFIIB. TFIIF together with Pol II are recruited and promoter DNA becomes tightly wrapped around Pol II. The entry of TFIIE into the complex is necessary for the accurate positioning of TFIIH [18]. Double-stranded DNA wrapped around is partially denatured closely upstream of the TIS, creating platform for binding of XPB subunit of TFIIH. ATP hydrolysis by the XPB ATPase induces conformational change in the protein that pulls one DNA strand out of the DNA helix [19]. Proposed mechanism of promoter melting by the XPB originates from the so called inchworm model [20]. The result is promoter melting in positions -9 to +2 around TIS [19]. Initiation of transcription is accomplished by the formation of the first phosphodiester bond in the new mRNA. In addition to its role in initiation of transcription TFIIH also plays a role in transition of Pol II from initiation to elongation. This switch is done by kinase activity of CDK7 subunit of TFIIH. Phosphorylation of CTD of the Rpb1 subunit of Pol II by CAK definitively shifts Pol II to elongation [21]. Pol II releases all general transcription factors except of TFIIE. Moreover, TFIIH plays essential role in RNA polymerase I transcription of ribosomal genes [22].

TFIIH and nucleotide excision repair. Nucleotide excision repair (NER) removes different structurally unrelated DNA helix-distorting lesions, including ultraviolet light (UV)-induced lesions and bulky chemical adducts. NER involves concerted action of ~25 proteins in a coordinated multi-step process including recognition of DNA lesions, unwinding the surrounding DNA, excision of the lesion and then filling in the resulting gap [23]. DNA lesions spread over the genome are targets for global genome NER (GG-NER), initiated by the XPC/hHR23B complex. Lesions that block RNA polymerase II elongation (i.e. transcription) are signaled directly by the presence of stalled RNA Pol II in the process known as transcription-coupled NER (TC-NER) [24]. After initial recognition TFIIH is recruited to the site of lesion and p8 subunit becomes stronger associated with TFIIH [25]. Subsequent p8-dependent recruitment of XPA leads to XPA driven detachment of the CAK from the core, thus transiently switching TFIIH from transcriptionally active to NER-engaged

[15]. Interaction of p8 with p52 subunit stimulates the ATPase activity of XPB. The mode of action of XPB is supposed to be similar in both the opening of DNA at the promoter and at the site of the lesion - large conformational change upon ATP hydrolysis allowing for local, but essential destabilization of DNA helix. It was shown that DNA opening in NER depends on the ATPase, but not on the helicase activity of XPB in combination with the processive helicase activity of XPD [26]. Expansion of the DNA bubble around the damage is facilitated by replication protein A (RPA). Single-strand to double-strand junctions are then targeted by structure specific endonucleases xeroderma pigmentosum G (XPG) and excision repair cross-complementing 1 - xeroderma pigmentosum F (ERCC1-XPF), thereby generating 3'- and 5'-incisions. The damage containing fragment excised by NER in mammals is about 25 - 30 nucleotides in length. The gap is filled by DNA polymerases δ and/or ϵ , DNA ligase I closes the 3' nick in the final ligation step [27].

TFIIH and cell cycle regulation. CDKs play central role in cell cycle control. Complete activation of a CDK requires cyclin binding and phosphorylation of a threonine residue adjacent to the kinase active site. Phosphorylation at this site is catalyzed by enzyme called CAK, which has constant level of activity in the cell. CDK7/cyclinH/MAT1 forms CDK activating kinase complex, which is responsible for the activating of phosphorylation of CDK1, CDK2, CDK4, and CDK6 [28]. All these kinases are important for G1/S and G2/M phase transitions of the mammalian cell cycle.

Recently it is not clear whether it is the intact TFIIH that is needed for cell cycle regulation or it is the released CAK only. Experiments with *Drosophila* [29] revealed sub-cellular distribution of CAK sub-complex and core TFIIH during early *Drosophila* embryo development. TFIIH core and CAK sub-complexes are initially located in cytoplasm. After the tenth mitotic divisions and until the cellular blastoderm stage, core moves to the nucleus but CAK is mostly cytoplasmic during cellularization and gastrulation. After this phase of development, CAK moves to nucleus and co-localizes with the core in most regions. Smaller part of CAK is localized in some DNA region alone without core TFIIH suggesting that CAK complex could have a TFIIH - independent role in transcription. Part of CAK that remains cytoplasmic probably stays for cell cycle regulation. It is known that the activation of CDK2/cyclin A and CDK2/cyclin B requires CDK7 [30] so it seems that the main function of CAK in the cytoplasm may be regulation of CDK2 activity. In accordance with this CDK7 activity is regulated by the level of XPD. In fact the excess of XPD results in cell cycle block at G2/M and decrease in XPD causes increased CAK activity and proliferation [31]. XPD recruits CAK sub-complex to TFIIH for basal transcription. At the beginning of mitoses the XPD is down-regulated. Degradation of XPD causes disengagement of CAK sub-complex from TFIIH probably for function in cell cycle control. This data suggests that CAK sub-complex acts as cell cycle controller without the core sub-complex of TFIIH.

However, the experimental data of Matsuno et al. suggest the involvement of the TFIIH holocomplex in cell cycle regulation. They studied the *Drosophila* eye imaginal disc, which is a very good model for monitoring TFIIH functions *in vivo* [32]. p52 subunit of TFIIH is required for CDK7-dependent cell cycle regulation. In this study researchers revealed that it is XPB that is also required for the CDK7 phosphorylation. Mutations in p52, XPB and CDK7 cause the same phenotype - block in the G1/S transition (G1 arrest) during developmentally-regulated cell cycle progression. This suggests that *in vivo* intact holocomplex of TFIIH is required for regulation of cell cycle. In fact CDK4 is more effectively phosphorylated by TFIIH complex than separate CAK sub-complex [33] supporting the need of the whole TFIIH in this process.

Human hereditary disorders associated with TFIIH.

Xeroderma pigmentosum. Mutations in XPB and XPD subunits of TFIIH, that affect only the NER activity give rise to "DNA repair disorder" xeroderma pigmentosum (XP) [34]. Faulty repair of ultraviolet DNA damage makes them extremely sensitive to UV light. Persistence of unrepaired lesions results in accumulation of mutations leading to cancer. XP patients are sun-hypersensitive, following sun exposure. XP patients develop excessive pigmentation and actinic keratoses on exposed skin. XP is associated with more than 1000-fold elevated sun-induced skin cancer risk, mean age of cancer development is less than 10 years. About 20% of XP patients have a progressive neurological degeneration in addition to the skin abnormalities.

Cockayne syndrome. Particular XPB and XPD mutations cause combined syndrome displaying the features of both XP and Cockayne syndrome (CS) [35]. CS is progressive neurological degeneration, characterized by retardation of growth and development after birth, cachectic dwarfism, microcephaly, deafness, ocular abnormalities, loss of subcutaneous fat tissue, hypogonadism, neural defects, demyelination and white matter degeneracy. Although CS patients are often normal at birth, the average lifespan of patients with CS is only ~12 years. At the cellular level, CS is defined by a failure to recover gene transcription after exposure to DNA damage, nevertheless the CS symptoms suggest overall abnormality in transcription.

Trichothiodystrophy. Third syndrome associated with mutations in p8, XPB and XPD subunits of TFIIH is trichothiodystrophy [36]. The hallmarks of TTD are sulfur deficient brittle hair (with characteristic alternating dark and light banding appearance under microscope) associated with neuroectodermal symptoms. Common features may include ichthyosis, skin photosensitivity, hypomyelination, mental and growth retardation, nail abnormalities, decreased fertility, short stature, cataracts, recurrent infections. In contrast to what occurs in CS, progressive neurological degeneration was not reported in TTD patients.

Mutations in TFIIH subunits found in TTD patients result in destabilization of the entire TFIIH complex. Recent studies using the TTD mouse model indicate that TFIIH acts as a co-activator for thyroid hormone dependent gene expression in the brain [37]. Reduced steady-state levels of TFIIH can prevent the regulation of hormonal responses, leading to TTD phenotype.

In conclusion, TFIIH is a typical example of the protein complex involved in a variety of metabolic pathways – from transcription and DNA repair to cell cycle regulation. The particular mutations in any of its ten subunits lead to cancer, premature aging, neurodegeneration or their combinations. The source of different mixed clinical features may be protein complex architecture – due to the direct protein-protein interactions of the subunits even a slight modification of one of them may influence the stability and functionality of the whole TFIIH.

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