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Envisioning how the prototypic molecular machine TFIIH functions in transcription initiation and DNA repair☆,☆☆

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ABSTRACT

Critical for transcription initiation and bulky lesion DNA repair, TFIIH provides an exemplary system to connect molecular mechanisms to biological outcomes due to its strong genetic links to different specific human diseases. Recent advances in structural and computational biology provide a unique opportunity to re-examine biologically relevant molecular structures and develop possible mechanistic insights for the large dynamic TFIIH complex. TFIIH presents many puzzles involving how its two SF2 helicase family enzymes, XPB and XPD, function in transcription initiation and repair: how do they initiate transcription, detect and verify DNA damage, select the damaged strand for incision, coordinate repair with transcription and cell cycle through Cdk-activating-kinase (CAK) signaling, and result in very different specific human diseases associated with cancer, aging, and development from single missense mutations? By joining analyses of breakthrough cryo-electron microscopy (cryo-EM) structures and advanced computation with data from biochemistry and human genetics, we develop unified concepts and molecular level understanding for TFIIH functions with a focus on structural mechanisms. We provocatively consider that TFIIH may have first evolved from evolutionary pressure for TCR to resolve arrested transcription blocks to DNA replication and later added its key roles in transcription initiation and global DNA repair. We anticipate that this level of mechanistic information will have significant impact on thinking about TFIIH, laying a robust foundation suitable to develop new paradigms for DNA transcription initiation and repair along with insights into disease prevention, susceptibility, diagnosis and interventions.

1. Introduction

TFIIH is an amazingly sophisticated and exemplary protein machine required for three key pathways in the cell: transcription initiation, global genome nucleotide excision repair (GG-NER) and transcription-coupled nucleotide excision repair (TC-NER) [1]. Depending on context, its composition changes from a core of seven subunits (XPD, XPB, p62, p52, p44, p34, p8) to ten subunits, by adding in a three subunit Cdk-activating-kinase (CAK) module (CDK7, cyclin H, MAT1). It utilizes three enzymatic activities at different pathway steps: 1) a

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serine/threonine kinase (CDK7), 2) a double strand (ds)DNA translocase (XPB), and 3) a 5′–3′ helicase (XPD) [2]. An SF2 helicase family enzyme XPB was originally identified as a helicase with limited *in vitro* helicase activity. As we note in detail below, recent XPB structures and biochemistry is more consistent with XPB being a translocase than a helicase, so we herein refer to XPB as a translocase. Furthermore, TFIIH regulates other enzymes including the structure-specific nucleases XPF-ERCC1 and XPG in NER, and RNA polymerase II (Pol II) in transcription initiation. Mutations in TFIIH are linked to three human diseases, xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy, which display distinct phenotypes [2]. The XPD subunit of TFIIH contains a [4Fe-4S] cluster, making it a member of a special group of DNA-binding [4Fe-4S]-containing proteins that includes DNA polymerases, helicases, primases, and glycosylases [3]. Overall, TFIIH is an essential, highly coordinated molecular machine whose precise orchestration of its functions and molecular mechanisms are only now being unveiled.

In transcription initiation, TFIIH opens the promoter DNA for Pol II and facilitates promoter release (reviewed in [4–6]). TFIIH is recruited as a ten-subunit complex (core plus kinase module) to Pol II preinitiation complex (PIC) by TFIIE (Fig. 1) [7]. Although TFIIH has two SF2 helicase family enzymes, only XPB (encoded by the excision repair cross-complementation group 3 gene, ERCC3) translocase activity is required to open up the promoter for Pol II to begin transcription [8]. When bound to TFIIH, CDK7 of the CAK kinase module phosphorylates the Pol II C-terminus to allow promoter escape. The free form of CAK activates the cell cycle CDKs, and the CAK-XPD complex regulates cell cycle progression in mitosis [9,10].

The TFIIH core (7 subunits excluding the kinase module) is essential for NER (reviewed in [2,11,12]), the only major DNA repair pathway for photolesions induced by ultraviolet radiation (UV) in placental mammals [13]. Strong sunlight can induce ~100,000 UV lesions per exposed cell per hour [14]), making NER a critical pathway. In fact, many TFIIH associated proteins were named through elegant genetic complementation experiments for xeroderma pigmentosum (XP), a rare inherited skin cancer prone disorder characterized by a heightened sensitivity to UV DNA damage [15–17]. These key genetic experiments make TFIIH an exemplary system not only for characterizing the interplay of repair with transcription and cell cycle, but furthermore, for linking molecular mechanisms to eventual prediction of biological outcomes from single site mutations [2,18–20].

Importantly, in addition to UV irradiation, NER repairs bulky DNA damage formed by environmental agents and cancer chemotherapeutic drugs. It operates through a multi-step process: lesion recognition, opening of the DNA around the lesion, lesion verification, excision of the lesion as part of an oligonucleotide, repair synthesis to replace the removed DNA and ligation to seal the nick (Fig. 2). GG-NER and TC-NER are two distinct repair sub-pathways and differ in their damage recognition. GG-NER is activated by recognition of destabilization of the DNA helix induced by the lesion, TC-NER by a stalled RNA polymerase II (Pol II) during transcription [21]. In GG-NER, the primary protein to recognize bulky DNA lesions, XPC-RAD23B, binds to DNA opposite to the lesion, which displays single strand (ss)DNA character, and unwinds the duplex to form a small bubble of ~ 2–3 nucleotides (nts) (**step 1**). Recent single molecule studies have shown that XPC-RAD23B may undergo diffusion around the lesions site until it fully engages with the lesion [22,23]. Centrin, a third member of the XPC complex, enhances XPC activity but is not essential in reconstituted NER reactions [24]. Another NER protein, the UV–DNA damage binding (UV–DDB) complex (not shown) is required for recognition of certain lesions, especially in the context of chromatin, reviewed in [25]. Once this small bubble has formed, XPC-RAD23B recruits TFIIH (**step 2**), which uses XPB translocase and XPD (encoded by ERCC2 gene) helicase activities to further open the duplex to create a bubble of 23−25 nts (**step 3**). Unlike for transcription initiation, both XPB and XPD ATPase activities are required for NER [26].

The XPA and RPA scaffolding proteins bind to this opened bubble [27]. XPA acts in damage verification for both GG-NER and TC-NER, and helps recruit the structure specific endonuclease XPF (ERCC4). So, if XPA levels are substantially decreased, it can become a rate-limiting factor for NER [28]. RPA serves to protect the ssDNA on the undamaged strand (**step 4**). XPF-ERCC1 and then XPG incise the dsDNA close to the 5′ and 3′ ss/dsDNA junctions of the DNA bubble, respectively; the lesion is thus removed as part of a ~25−27mer excised oligonucleotide [29–31]. The dual incision step is a highly coordinated process, and current models suggest that ERCC1-XPF makes the first incision (**step 6**), followed by partial repair synthesis (**step 7**), incision 3′ to the lesion by XPG (**step 8**) and completion of repair synthesis and ligation. DNA Pol δ (and possibly other polymerases), RFC, PCNA, and DNA ligase I or IIIα are involved in these later NER steps (**step 10**).

XPC-RAD23B and UV-DDB are not involved in the TC-NER subpathway, and bulky DNA damage in transcribing strands is instead efficiently recognized by the lesion stalling elongating RNA Pol II (reviewed in [11,21]). This triggers ubiquitylation of RNA Pol II at a single residue (K1268) and initiation of TC-NER [32,33]. CSB is then recruited, followed by CSA and its associated DDB1-CUL4 ubiquitin

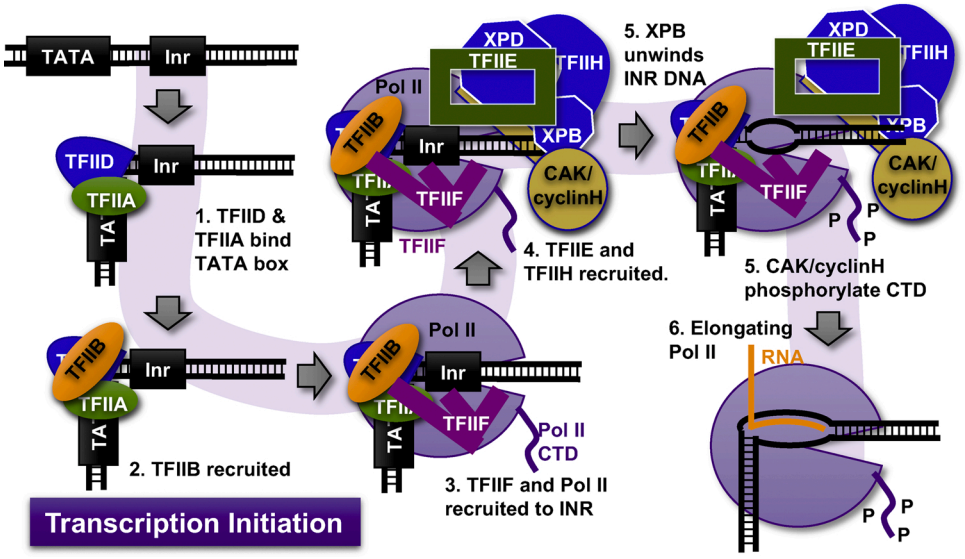

Fig. 1. Pathway for Transcription Pre-Initiation Complex (PIC) and Initiation. After TFIID, TFIIA, TFIIB recruit Pol II, TFIIE is recruited and in turn, recruits TFIIH. TFIIE is positioned as a molecular latch, locking down TFIIH and limiting overall TFIIH flexibility. The XPB translocase will negatively supercoil the initiator element (INR) DNA to open it for Pol II. Pol II C-terminal domain (CTD) phosphorylation by the CAK kinase module in the ten-subunit TFIIH will promote Pol II release from the INR.

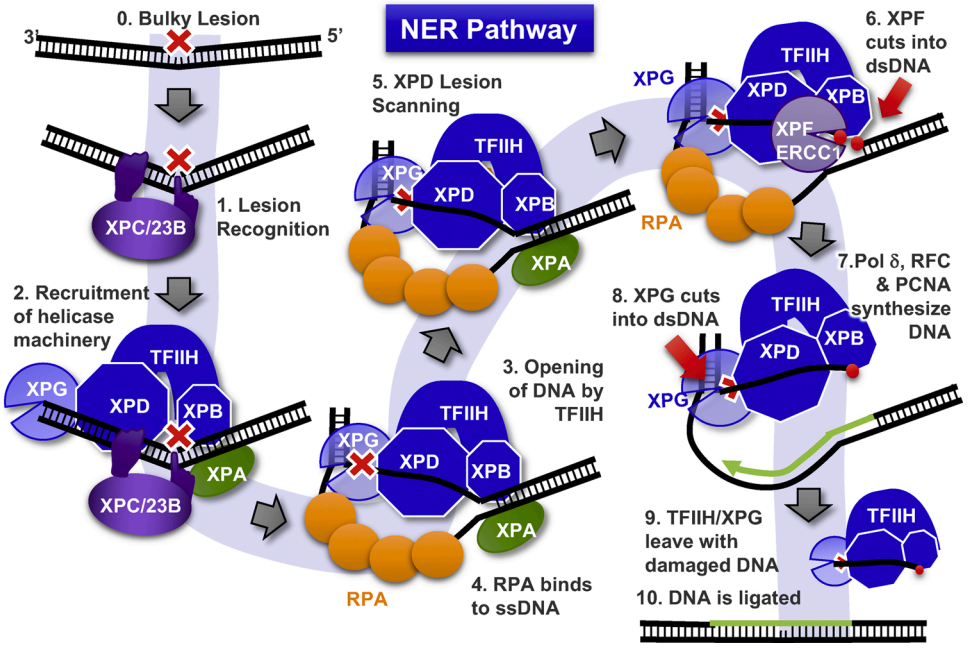

Fig. 2. Pathway for Nucleotide Excision Repair (NER). 0) Bulky DNA lesions are repaired by NER. (1) In global repair, XPC-RAD23B recognizes the lesion (red X). (2) Helicase complex machinery (TFIIH, XPG, XPA) is recruited by XPC. (3) XPB subunit of TFIIH translocates along the DNA, leading to formation of ssDNA. (4) XPC-RAD23B, whose primary interface is to dsDNA, is released. RPA binds and protects ssDNA on the undamaged strand. (5). XPD captures the ssDNA and uses its ATPase activity to find the lesion. (6) XPF makes the first cut (arrow) in the dsDNA 5′ to the lesion. (7) Polδ, RFC, and PCNA load onto DNA 5′ to the lesion and start DNA synthesis replacing the damaged region. (8) XPG incises the dsDNA 3′ to the lesion (arrow). (9) TFIIH and XPG leave with the damaged strand. (10) To complete repair, DNA ends are rejoined by DNA ligase.

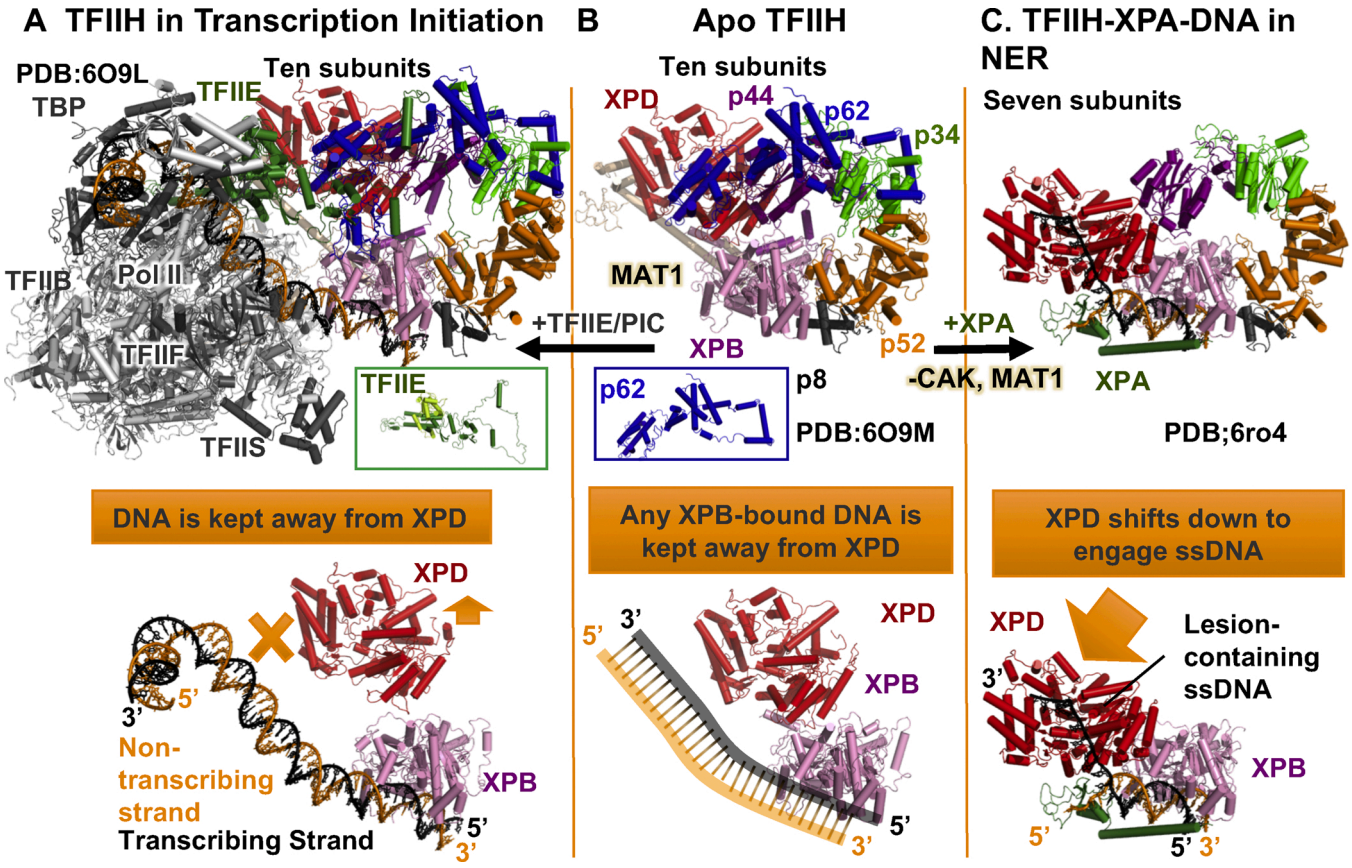

Fig. 3. TFIIH functional conformational changes enable differentactivities. Structures are overlaid based on the XPB chain and shown as ribbons. Upper panels show TFIIH A) in a transcription PIC complex with promoter DNA, B) in solution and DNA-free, and C) in an initial NER complex with XPA and Y-DNA. All models shown are based on cryoEM density, and subunits or subunit regions not detectable in density are not shown. Thus, PIC and solution TFIIH models are missing CAK/cyclin H subunits present in the original sample, while the TFIIH-XPA-DNA model is missing p62. The PIC and solution models are additionally based on our integrative computational methods. Pol II and transcription factors are colored gray. Insets highlight molecular rigging character of TFIIE and p62. Lower panels show only the DNA, XPB, XPD, and XPA chains. In transcription initiation, XPD is shifted away from the DNA (marked by small arrow and X). In NER, XPD rotates towards and binds the ssDNA (marked by large arrow). Scale and perspective are maintained in all panels.

ligase complex [33,34]. CSA and CSB together promote the association of the UVSSA protein, which is in turn ubiquitylated and required for TFIIH to join the TC-NER complex [33,34]. Although the fate of ubiquitylated RNA polymerase during TC-NER is not fully understood, current models suggest that it is removed from the damaged strand, allowing for TFIIH to fully engage the lesion. A striking similarity between GG-NER and TC-NER is that XPC and UVSSA share an interaction surface in the pleckstrin homology (PH) domain of the p62 subunit of TFIIH, suggesting that the two pathways at least partially share a mechanism for the engagement of TFIIH with the lesion site [35,36]. Given that XPA, RPA, XPG and ERCC1-XPF are also required for TC-NER, and that the size of the excised oligonucleotide is similar, following engagement of TFIIH, the mechanism for TC-NER repair is thought to closely resemble GG-NER. Interestingly, an added component for NER in lower eukaryotes - the ATL1 (alkyltransferase-like 1) protein – has been shown to regulate choice between GG-NER and TC-NER on the basis of how tightly it binds to alkylated DNA, which in turn appears to depend on the size of the lesion [37,38].

The mechanism for how TFIIH opens dsDNA remains an enigma. While both XPD and XPB have homology to the SF2 helicase family and display limited helicase activity, their unwinding and opening of dsDNA is more consistent with a model where they function as ssDNA and dsDNA translocases whose activities lead to strand separation, respectively. Building upon crystal structures of TFIIH subunits, breakthrough cryo-electron microscopy (cryo-EM) structures of TFIIH complexes have provided informative glimpses of TFIIH alone, and as it would appear in transcription initiation and in NER (Fig. 3) [39–44]. Excellent reviews cover the details of these structures, along with insights into specific activities and structural mechanisms [4–645–47].

Here we build upon knowledge of TFIIH evolution, genetics, and structures, combining them to address how TFIIH works, *i.e.* the underlying mechanisms for its biological functions. Our premise is that while genetics and biochemistry provide deep insights into what the individual TFIIH subunits do, structures can help us to understand how they do it. Importantly, as these proteins have been perfecting their activities for three billion years, they have much to teach us about how they accomplish their amazing efficiency and specificity. This information in turn provides clear direction for future research and better understanding of NER biology and medicine.

We consider the following critical questions. How does TFIIH work? What are the differences between TFIIH mechanisms in transcription initiation *versus* DNA repair? What can we learn from the genetics and disease mutations? This perspective builds upon the field's current knowledge and highlights what we know, points we infer, and mechanisms that remain to be explored. Since biology works by the bottom-up self-assembly of proteins from sequence building blocks to develop complex functionalities, we will try to place integrated information and models into context of sequence-based atomic models. Our objective is that these atomic-level models stimulate ideas and discussions, identify important unanswered questions, and suggest future experimental directions for the scientific community.

2. Overall architecture of TFIIH

To begin this discussion, we first introduce the overall architecture of TFIIH and then the three "types" of TFIIH structure (Fig. 3). A) TFIIH (10 subunits) as part of the Pol II PIC in transcription initiation [40,42,43,48,49]; B) TFIIH (10 subunits) alone [39,43,44]; C) TFIIH (7 subunits) with XPA and Y-DNA [41]. Supplemental Movie 1 highlights conformational changes between the three types of TFIIH.

TFIIH has a central core of 7 subunits (Fig. 3C) [50–54]. XPB [55,56] and XPD [57–59] each have RecA/Rad51 helicase domains 1 and 2 (HD1 and HD2). XPD has an additional two domains, an [4Fe-4S] cluster domain (FeS) and an arch domain whose sequences are interwoven with HD1. XPB has poor helicase activity *in vitro* and is likely a dsDNA translocase that results in strand separation, in concert with other proteins. XPD has limited helicase activity requiring 5′ ssDNA overhangs and is implicated in verifying the presence of a chemical modification after initial recognition of helix destabilization by XPC [57–63]. The five other subunits have no known enzymatic activity and are named on the basis of their approximate molecular weight: p8, p34, p44, p52, and p62. With the exception of p62, these subunits are globular and assemble into a horseshoe shape with XPB and XPD on the ends. p62 is an extended, non-globular protein that stretches out like molecular rigging, connecting 5 of the 7 subunits (XPD, XPB, p44, p34, and p52). Interestingly, its path switches between p52 and XPB depending on if TFIIH is free in solution or in a transcription initiation complex, respectively. The p44 subunit is surrounded by p34, XPD, p62, but also stretches out N- and C-terminal helices to contact XPB, fully consistent with its critical role in TFIIH assembly. The kinase module adds another three subunits in transcription initiation: CDK7, Cyclin H and MAT1. Unlike CDK7 and Cyclin H which are thought to be flexibly tethered to the core and thus have not been modeled in the cryo-EM structures, the MAT1 subunit acts as a brace between XPB and XPD and likely reduces XPD domain mobility [64].

Analysis of the three TFIIH conformations (Fig. 3) reveals how evolution has structurally optimized TFIIH to avoid potential problems arising from its roles in both transcription initiation and NER, which might occur if TFIIH inadvertently switches from transcription initiation to NER and the mistakenly initiated repair process causes ssDNA breaks. Similarly, TFIIH can be toxic to the cell as it could randomly bind and open up dsDNA. Thus, TFIIH uses multiple controls to regulate XPD activity. 1) *The XPB-bound dsDNA path is away from XPD.* In transcription initiation, the configuration of TFIIH results in the dsDNA path leading away from XPD and towards Pol II (Fig. 3 lower panels). A similar dsDNA trajectory can be extrapolated from the XPB position in the solution structure. If the dsDNA base stacking is disrupted as observed from XPC-RAD23B insertion at the DNA lesion in NER [65], the dsDNA could be bent to the extent needed to redirect its path towards XPD. This suggests that an external protein is required to license XPD activity. 2) *XPB is positioned away from XPD.* XPB is physically separated from XPD in free TFIIH and in transcription initiation complexes, compared to the TFIIH-XPA-DNA NER complex (Fig. 3 lower panels). Both MAT1 (in the CAK subunit) and XPA span XPB and XPD, but with opposite consequences. The long MAT1 helix is reminiscent of a spanner that separates XPB and XPD, while the arms of XPA, with MAT1 removed, appear to facilitate bringing XPB and XPD together. 3) *Blocking of XPD helicase sites.* In the free TFIIH and PIC structures, the XPD ssDNA-binding groove is blocked by p62 and XPB, and the XPD ATP-binding site is capped by p62, likely inhibiting both DNA binding and helicase activity of XPD (Fig. 4A) [39,43]. Thus, the seemingly meandering path of p62 has real functional implications in free TFIIH and PIC complexes. In the TFIIH-XPA-DNA NER structure, p62 is not modeled in the density [41]. However, it has been proposed that some of the unassigned cryo-EM density is p62 aligned above the XPD ssDNA binding groove [66]. The strong enhancement of XPD helicase activity by XPA makes XPA the likely candidate for moving p62 and XPB out of the XPD functional sites [41,60]. 4) *Regulation of XPD mobility.* The action of SF2 helicase family members requires domains to move relative to each other. MAT1 interacts with the ARCH domain in XPD, which will reduce inter-domain motion in XPD, consistent with the reported reduction in XPD helicase activity [64]. We did not observe any such negative controls for XPB. Taken together, these observations illustrate that TFIIH quaternary structure is designed to be transcription initiation-ready, but repair-inhibited.

In contrast to the organization of TFIIH in the PIC and free structures, the TFIIH-XPA-DNA structure unveils the repair-ready conformation. In this structure, XPD has shifted unexpectedly by ~80 Å to be next to XPB and in position to bind the first ssDNA nts at the ss/dsDNA junction (Fig. 4B). Other subunits in the core TFIIH horseshoe also shifted and rotated to accommodate this arrangement. The "hole" in the TFIIH center, first speculated as a place for DNA binding, closes like a finger

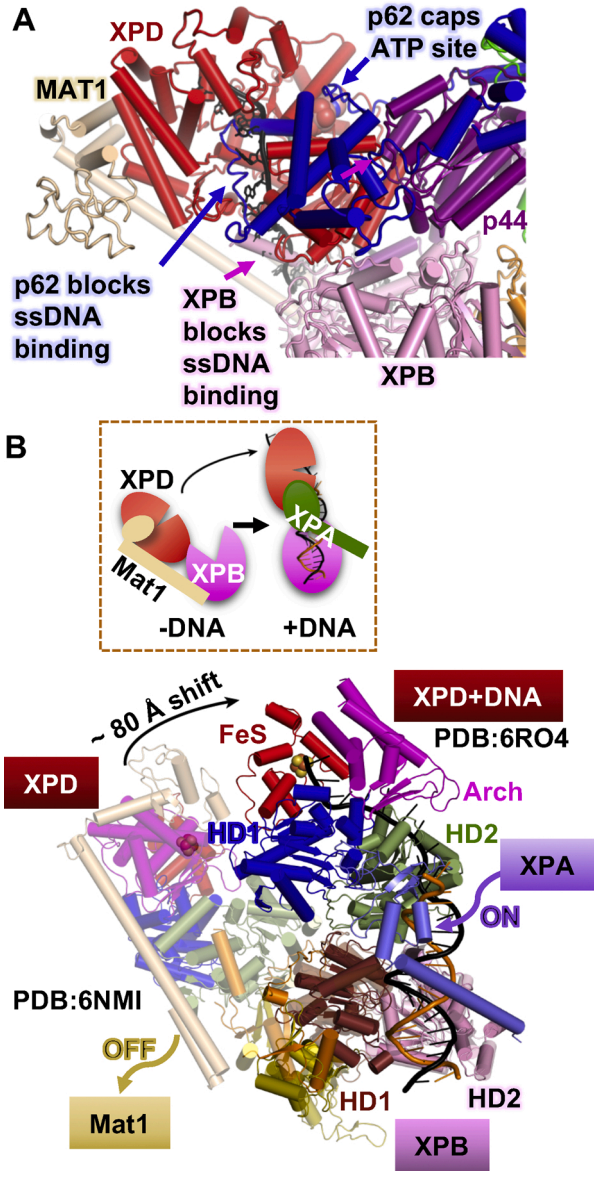

Fig. 4. TFIIH moves from transcription initiation-ready, repair inhibited to repair ready. A) TFIIH is repair inhibited. p62 and XPB block XPD ssDNA binding path; and p62 caps the ATP-binding site; and MAT1 prevents XPD domain motions. Overlay of apoTFIIH (protein ribbon, PDB:6O9M) with ssDNA (TFIIH-XPA-DNA, PDB:6O9M), and BeADP (spheres, DinG, PDB:6FWS). XPD HD2 domain was used for the overlay. B) Conformation changes between solution, DNA-free XPB-XPD (apoTFIIH) and DNA-bound XPB-XPD (TFIIH-XPA-DNA) subcomplexes (overlay on XPB). MAT1, a part of CAK subcomplex, is bound between XPB and XPD to restrict XPD from binding to DNA. To enable NER, XPA is recruited to bind XPB and dsDNA. XPA facilitates XPB unwinding through separating pin Trp175, disengages CAK subcomplex to allow XPD to shift ~80 Å to load on unwound ssDNA, and holds XPD close to 5′ end of the junction to start lesion scanning to 3′ end on the damaged strand.

trap in the TFIIH-XPA-DNA structure and in fact may be required for this rearrangement to occur (See Supplemental Movie). It will be interesting if differentially exposed surfaces on TFIIH subunits mediate protein-protein interactions in subsequent stages of NER. For example, XPB residues 315–328 are in the interface with XPD in the solution structure but are exposed in the TFIIH-XPA-DNA structure. Could these residues be important for XPB interaction with RPA and/or XPF? In the following we consider mechanisms for how XPB and XPD work.

3. How does XPB work as a dsDNA translocase to open dsDNA?

XPB was historically identified as a 3′–5′ helicase, even though it didn't work as a helicase in classical helicase assays that measure generation of ssDNA from dsDNA. It was also drawn as binding to ssDNA. In fact, as noted years ago [67,68] and recently confirmed [69], XPB is not a typical helicase but a translocase that moves along dsDNA and unwinds dsDNA, as a complex with other proteins. Working on duplex DNA, XPB binds both strands and does not show polarity.

Given this reassignment of XPB as a dsDNA translocase, it is worth revisiting conclusions from initial analyses of XPB as a "helicase" and from studies that use mutations that disrupt XPB's "helicase" activity. In particular, we re-examined the interpretation that XPB helicase activity is not required for NER [26]. The authors showed that mutants within one XPB helicase motif caused partial reduction in transcription initiation and no apparent impact on NER, from which it was concluded that XPB "helicase" activity is not required for NER, only the ATPase activity. With the hindsight provided by XPB structures with DNA [41,42], we found that the "helicase" residues, mutated in that study, do not directly contact DNA and predict that the mutants likely reduce, but do not eliminate XPB domain movements and translocation. If XPB translocation was not rate limiting in their NER assay, reduced activity could not have been detected, putting back into question if XPB translocase activity is required or not in NER. Mutations designed from the currently available structures should enable generation of DNA binding-defective XPB variants that will make it possible to distinguish whether the required ATPase activity is needed in NER for the translocase activity or for allosteric communication. In the meantime, we hypothesize that XPB translocase is involved in NER.

So, how does the XPB translocase lead to unwound DNA? We propose two complementary mechanisms.

With XPB and XPD somewhat fixed relative to each other, it is important to change the perspective for thinking about the action of these enzymes. Classically, translocases and helicases move relative to DNA, which is held stationary. This makes sense in a chromosomal context as the DNA would be less mobile than the protein. With respect to the lesion-containing strand, XPB is a dsDNA translocase that moves 3′–5′ and XPD a helicase that moves 5′–3′. However, XPB and XPD are held in relatively fixed positions in TFIIH complexes; they cannot move in opposite directions on the same DNA while remaining roughly at the same distance from each other. If the DNA is moving, then this apparent paradox is resolved. This is also applicable in transcription initiation (see discussion below). So, to understand our proposed mechanisms, we change the perspective to DNA being moved by a mostly fixed XPB and XPD.

The first mechanism is applicable for both transcription initiation and NER. XPB acts by twisting dsDNA inducing negative supercoiling, thereby opening the duplex [43,48] (Fig. 5). We first describe features of this mechanism for the PIC in transcription initiation. 1) XPB binds in the minor groove, as observed in TFIIH structures with DNA. 2). We assume that XPB remains in the minor groove as it translocates, analogous to a unicycle (XPB) on a track (DNA minor groove). 3) We further assume that XPB is fixed, which means that the DNA moves. Because DNA is helical and XPB stays in the minor groove, the DNA spirals towards Pol II and the other PIC factors. 4) XPB is locked to Pol II and the PIC by TFIIE and by subunits of TFIIH such as MAT1 and p62 [43]. Therefore, XPB and Pol II do not move relative to each other. 5) The DNA bound in the PIC is fixed and cannot rotate. 6) Because the PIC side of the DNA is fixed, the spiraling DNA moving away from XPB cannot relieve tension and becomes negatively supercoiled [67,69,70]. 7) Negative supercoiling causes DNA unpairing [71].

In GG-NER where there is no Pol II, other NER proteins prevent the DNA from freely rotating as XPB translocates: XPC-RAD23B, XPA, p62, and XPD are all candidates. The requirement is that they prevent DNA rotation on the other side of the targeted region, while being fixed relative to TFIIH. Early on in NER, it is likely that XPC-RAD23B prevents

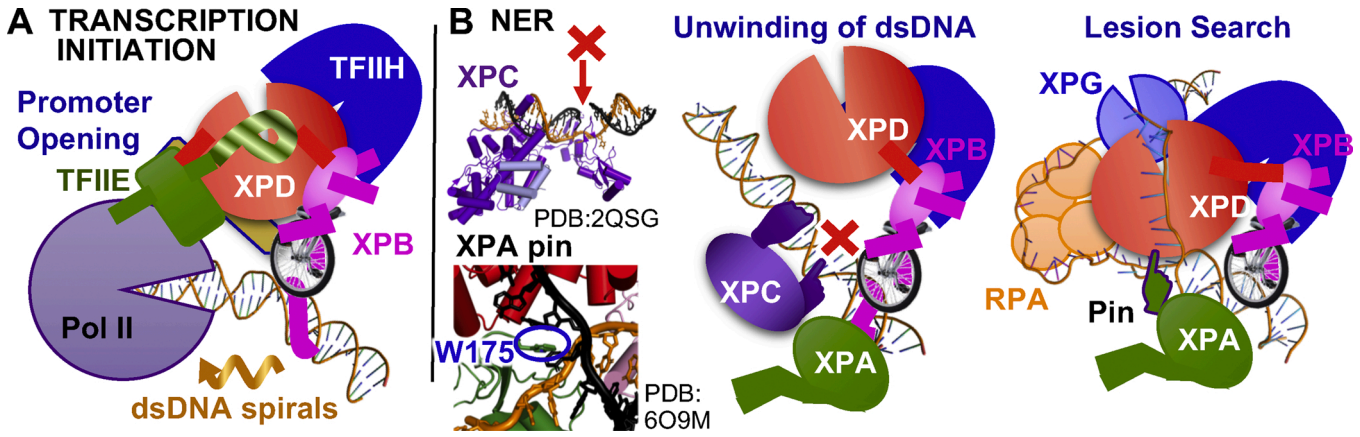

Fig. 5. Envisioning how XPB as a translocases negatively supercoils DNA to unwind it. Cartoons of XPB translocase, represented as a unicycle riding in the minor groove of dsDNA. TFIIH is represented in blue, red (XPD),magenta (XPB) and tan (MAT1). (A) In transcription initiation, TFIIE and MAT1-based girded protein-to-protein connection locks XPB to Pol II. With Pol II and other transcription factors gripping the dsDNA, XPB translocates in the minor groove, compels the dsDNA to spiral right-handed towards Pol II, induces negative supercoiling, and opens the promoter element. (B) In early stage NER, XPC-RAD23B, and XPA could fix the dsDNA relative to XPB. Insets) XPC-RAD23B binds dsDNA 3′ to the lesion (X) and the two bases opposite to lesion. XPA both" braces" the ssDNA against XPB and contributes a pin (Trp175) that helps separate the strands. XPC-RAD23B and/or XPA prevents rotation as XPB translocates in the minor groove. The DNA spirals towards XPC-RAD23B becomes negatively supercoiled and unwinds. Later, XPD captures the newly formed ssDNA, and XPG the dsDNA, in place of XPC.

rotation of DNA; XPC binds to duplex DNA non-specifically through the TGD-BHD1 domains and begins unwinding the DNA by inserting its BHD2 and BHD3 loops into the DNA duplex at the lesion site [65] (Fig. 5B) [65,72]. XPC insertion and opening of the duplex DNA is energetically linked to DNA bending, a feature that can help redirect the dsDNA towards XPD, as in Fig. 3C. XPC has at least two interaction surfaces with TFIIH –one at the C-terminus believed to be involved in binding XPB and the other, a region at the N-terminus that binds the p62 pleckstrin homology (PH) domain [35,73]. We propose that the multiple interaction sites of XPC with DNA, p62 and XPB could anchor XPB, thereby preventing free rotation of the DNA. Consistent with this idea, DNA opening is increased upon addition of TFIIH and ATP to XPC [74]. This opening of the DNA could be sufficient to allow XPD to bind to ssDNA (preventing rotation and as part of TFIIH, being fixed to XPB). In the TFIIH-XPA-DNA structure, XPA inserted its Trp175 on a beta hairpin at the ss/dsDNA junction and would thereby prevent rotation, while being fixed against TFIIH (Fig. 3C). The XPD arch domain, p44, and p62 are also implicated in DNA binding and are therefore candidates to prevent dsDNA rotation [64,66].

As the bubble is progressively opened and other proteins are recruited, XPC is released and would no longer contribute to negative supercoiling. XPG recruitment is coincident with XPC displacement [75–78], either due to competition or conformational changes that eliminate the XPC binding interface to TFIIH. Could enhancement of XPD helicase activity by XPG and disruption of XPC dsDNA binding interfaces by negative supercoiling and unwinding of the dsDNA partially contribute? Coming back to the question of the DNA tension from negative supercoiling, the previously XPC-occupied DNA is positioned to be clamped by XPD on the ssDNA and XPG on the dsDNA. Another possibility is that XPB translocase activity, perhaps after binding of XPA, induces additional supercoiling that together with the XPD translocation toward the lesion leads to dissociation of XPC-RAD23B.

The second mechanism is specific to NER and incorporates the departure of the CAK, in particular MAT1 which spans XPB and XPD. We propose that XPA has an important role. In the following, we describe the critical features of this mechanism. **1**) In the TFIIH-XPA-DNA structure [79], Trp175 in XPA is positioned to serve as a "strand-separation pin" (Fig. 5B) [41]. Thus, as XPB translocates, the dsDNA is split by the pin, as observed in many helicases [80–83]. **2**) XPA disengages the CAK kinase module from TFIIH [84], freeing the core TFIIH from MAT1. In the structures of free TFIIH and of the PIC-TFIIH, MAT1 acts as a brace to separate XPB and XPD (Fig. 3) [39,42,44]. XPD can move, such that the XPA pin is ideally positioned and the damage-containing ssDNA is immediately bound by the strongest DNA binding interface on XPD [41]. **3**) XPA traps the dsDNA in the XPB DNA binding groove and shifts p8 and XPD, promoting DNA-dependent ATPase activity [41,60,74,85]. Although elements of this mechanism are common with XPA in mechanism 1 (preventing rotation and fixing XPA to TFIIH), the critical difference is the strand-separation pin. Could the XPC opened dsDNA be sufficiently large for insertion of the XPA strand separation pin, Trp175, negating the initial need for negative supercoiling in Mechanism 1? Further mutagenesis and biochemistry studies are needed to parse out the relative contributions of mechanisms 1 and 2 for NER.

4. How does XPD open up the DNA?

In contrast to XPB binding to dsDNA, XPD binds ssDNA. As expected, the primary ssDNA path goes along the two XPD domains HD1 and HD2. Two important structures of the ssDNA-bound E. coli XPD ortholog DinG, with and without an ATP mimetic (ADP-BeF) shed light on the XPD helicase mechanism (Fig. 6A) [86]. HD1 and HD2 shift from being apart in the resting state (ssDNA bound, but no nt cofactor) to being closer together in the active state with an ATP-mimetic bound. Besides the interactions identified by Cheng and Wigley, we examined B-factors in the ssDNA bound by DinG; B-factors reflect the positional stability of each atom and provide an indirect, but quantitative measurement of binding stability (Fig. 6B and C), as shown for antibody-protein interactions [87]. Here the lower the B-factor, the more stabilized is the ssDNA. The resting DinG structure (without nt cofactor) appears to have tighter interaction at the 3′ and 5′ ends of the ssDNA, while the ATP mimetic-bound DinG has tighter interactions in the center, a region on HD2 near HD1. Strikingly, this central ssDNA region in the human TFIIH-XPA-DNA structure is lifted off from the bottom of the DNA binding groove [41], analogous to the DinG resting state. We define three changing ssDNA interaction points: HD1, HD2 Center, and 5′-HD2 based on their associated domain and relative position to 5′ and 3′ ends of the ssDNA.

We propose a multi-step model for XPD helicase activity, building on both the XPD structures and the Cheng and Wigley DinG insights [86] (Fig. 6D). 1) HD1 and 5′-HD2 bind to the ssDNA, with HD2 Center loosely bound, as observed in the DinG resting state. 2) ATP binding induces HD1 to bind the 3′ ssDNA end, shifting toward HD2 and moving the DNA 1 nt towards the 5′ end, as expected for a 5′–3′ translocase. 5′-HD2 releases its hold on the DNA; if it did not, then the central ssDNA

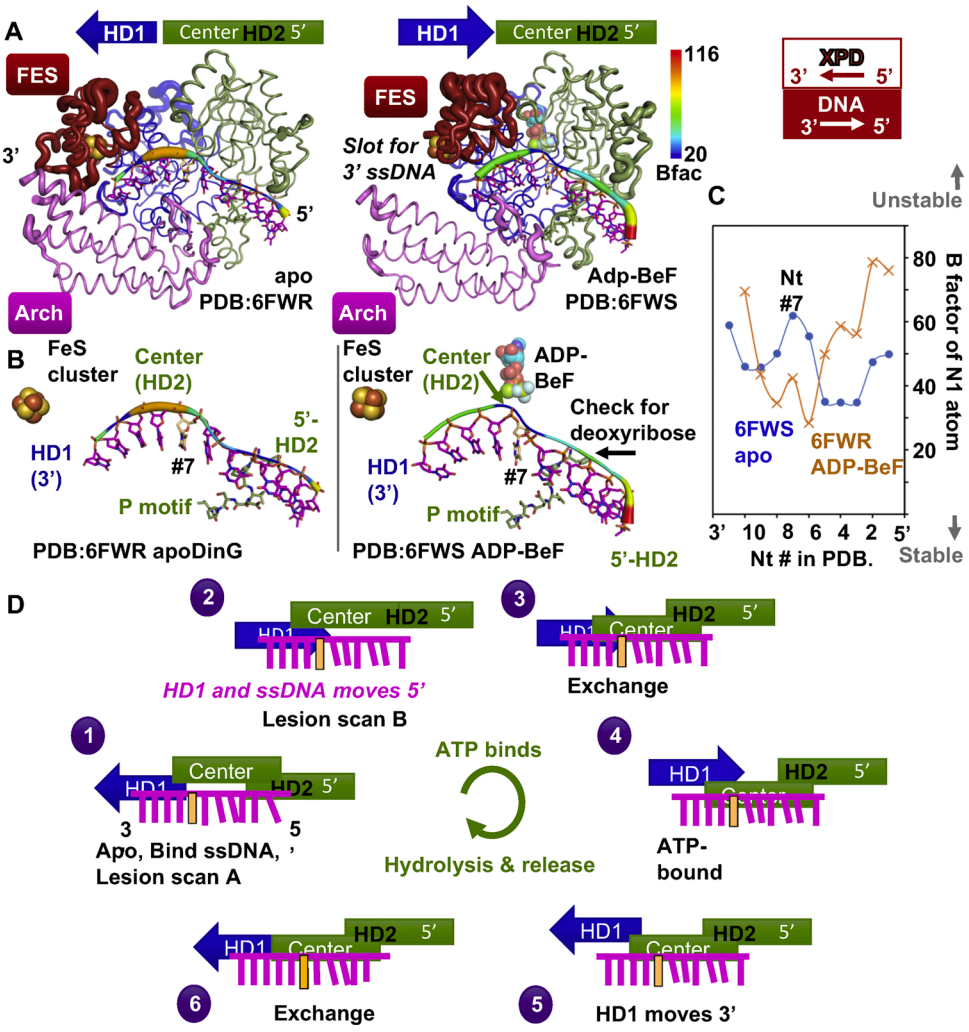

Fig. 6. DinG structures reveal structural elements underlying XPD helicase mechanisms, lesion scanning, ssDNA loading, and preference against RNA. (A) Ribbon representations of Apo and ADP-BeF-bound DinG structures show changes in domain organization and flexibility. Cartoon width is based on B-factor, an indicator of flexibility. DinG is colored by domain; ssDNA backbone, by B-factor. The ssDNA is also depicted as sticks, with nt 7 colored light orange for register. ADP-BeF and [4Fe-4S] are shown as spheres. (B) DNA, FeS, ADP-BeF, and proline in same perspective as A. (C) Plot of B-factor against the ssDNA nt reflects indirectly ssDNA stability. (D) Hypothesized HD1/HD2 multi-step mechanism for 5′–3′ helicase and lesion scanning. Overlay of domain boxes on ssDNA (sticks) represents tighter binding. Stacked DNA bases are drawn vertical.

would bend upward even more. 3) HD2 Center clamps down (preventing the ssDNA from moving back). 4) HD1 releases its hold on the ssDNA, as seen in DinG with ATP-mimetic bound. 5) ATP is hydrolyzed and released as ADP. HD1, not binding tightly to the ssDNA, shifts back towards the 3′ end. 6) HD1 and 5′-HD2 clamp down on the ssDNA. 7) HD2 Center releases the ssDNA, setting the protein back to the resting DinG state (Return to step 1). This multi-step process is likely regulated by conformational changes induced by binding of ATP, ATP hydrolysis, phosphate group release, and nt cofactor release and within TFIIH, through interactions with XPB and other TFIIH subunits.

5. How is ssDNA loaded onto XPD?

The strongest ssDNA binding interface of XPD is at the 5′-HD2 end. This is reflected in the binding to this region observed in the first XPD-DNA structures and by the severe impact of mutations in the 5′-HD2 region [57,86,88,89]. We hypothesize that it is not coincidental that the XPA "strand separation pin" is located next to the 5′-HD2, the strongest ssDNA binding region on XPD. Thus, the 5′-HD2 is the most likely candidate for initial loading of ssDNA, opened first by XPC and then enlarged by XPA and XPB. This ssDNA binding is likely to occur in the nt cofactor-free form, which showed the strongest DNA binding at the 5′ HD2, based on low B-factor in DinG. Furthermore, a corollary to above helicase model is that XPD will not function as a helicase until HD1 also binds to ssDNA: this sets a minimal 10–11 nt ssDNA length for XPD helicase action, the number of nts required to span 5′-HD2 to HD1.

The TFIIH-XPA-DNA and DinG structures revealed the 3′ end threading between the HD1, Arch, and FeS domains. However, ssDNA in an NER reaction is always in the context of a DNA bubble with no free end and therefore would need to be slotted through. The DinG structures show just such a slot formed by opening up a 10–15 Å gap between the FeS and Arch domains in the structure with ATP mimetic bound (Fig. 6A). The center clamp of DNA (lowest B-factor) by HD2 in the ATP-bound state may be a grip point to facilitate slotting through of the 3′ end. In both DinG structures, the FeS domain has the highest B-factor, suggesting the greatest flexibility.

6. How does XPD detect the lesion?

Evidence that XPD detected lesions came first from yeast studies and later with purified proteins that showed XPD helicase and ATPase activities are inhibited by lesions [60,62,90,91]. Archaeal XPD structures suggested how a lesion could be sterically blocked and thereby detected *via* a small opening in the protein at the end of a potential ssDNA binding groove [57–59].

In our mechanistic perspective, we first consider the substrate nature. The typical DNA lesions repaired by NER are helix distorting and bulky. XPD binds ssDNA so helix distortion is not relevant, but the bulky size of adducts would be detectable. Based on XPD structures, two regions could potentially detect different types of lesions (Fig. 7). In site A, first proposed in the initial XPD structures [57–59], a sequence-conserved narrow passage is formed by the HD1, FeS, and Arch domains. Site A is 7 Å at its most narrow. Lesions may be detected when the ssDNA moves 3′ concurrent with the DNA-unbound HD1 shift,

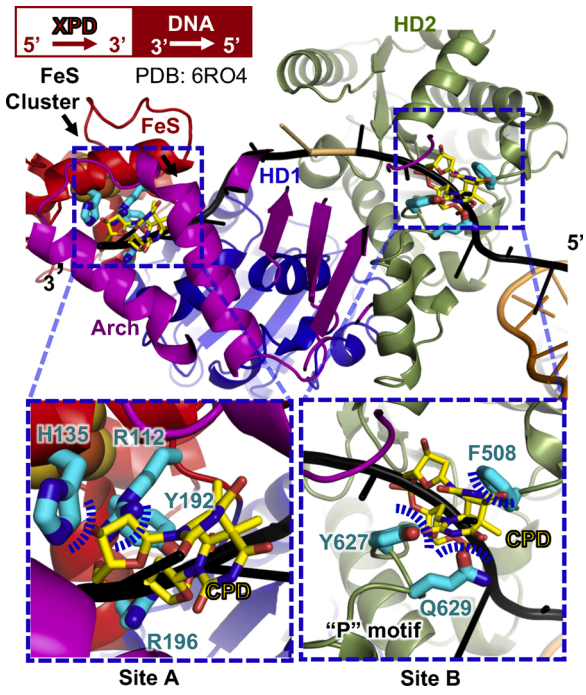

Fig. 7. Implicated Lesion Scanning Sites in XPD. Along the ssDNA binding path on XPD, there are two sites which would sterically clash the CPDs. Ribbon representation of human XPD with DNA, based on TFIIH-XPA-DNA structure with overlaid CPD (stick) at the putative lesion-detecting sites. Parts of XPD were removed by depth slicing for clarity. For register, the nt corresponding to nt #7 in Fig. 6 is colored light orange. Otherwise, the DNA is colored as in Fig. 3. Insets show Sites A and B, with CPD steric clashes (blue lines).

after ATP hydrolysis (step 5, Fig. 6D).

In site B, the ssDNA enters XPD as base stacked, but becomes distorted as it passes over a proline (P) motif, identified in DinG structures. The P motif is not sequence-conserved but is structurally conserved from bacteria to humans. Lesions that cause one or more nts to become rigid might be detected by their inability to pass over the P motif when the HD1-bound ssDNA moves 5′ upon ATP binding (step 2). Adding an additional measure to force the break in base stacking, the nt bound just 3′ to the P motif (colored light orange in Fig. 6) is the most stabilized in the DinG-bound ssDNA. Intriguingly, this nt is the one lifted up from the bottom of the DNA binding groove and may also be a contact point for p62 in the human TFIIH-XPA-DNA, based on unassigned density in the cryo-EM structure that is consistent with p62 also being involved in lesion detection [66]. With site B being 7 Å at its most narrow, XPD may use the ability of the ssDNA to maneuver between the HD2 center clamp and the P motif to scan for lesions that cannot, such as intra-strand cyclobutane pyrimidine dimer (CPD) or 6−4 pyrimidine-pyrimidone (6−4 PP) [86]. Indeed, it has been proposed that this P motif might sense the sugar hydroxyl in a ribonucleotide, shown to be an NER substrate [86,92]. Both site A and site B would require ATP-driven conformational shifts between HD1 and HD2, as biochemically required for lesion scanning [93].

There is experimental support for site A over site B, for scanning lesions. If XPD binds the ssDNA at the ss/dsDNA junction 5′ to the lesion, as seen in the most recent TFIIH structure in complex with XPA [41], there should be ~11 nt for Site A or 6 nt for Site B of ssDNA 5′ to the junction. Studies to detect opened ssDNA in extracts or with reconstituted NER reactions show $KMnO_4$ labeling 10−11 nt on the 5′ side of cisplatin (cisPt) DNA lesion, consistent with site A detection for that lesion [74,94]. Comparison of the structurally implied nt number on the 3′ side of the lesion to the $KMnO_4$ data is complicated by the likelihood that the ss/dsDNA junction is not directly binding XPD but rather is bound by the XPG nuclease. XPG, which makes the second cut in NER but binds before ERCC1/XPF, is a founding member of the FEN1/XPG 5′ nuclease superfamily that binds and sculpts ss/dsDNA junctions [31,95–100]. Yet, unlike the flap endonuclease FEN1, which must thread a DNA end to avoid genome instability [101], XPG can cut bubble DNA, so it must be able to slot ssDNA into its active site when bound to the ss/dsDNA [102]. Perhaps the strongest support for site A (and not site B) being critical for lesion detection comes from the finding that mutation of site A residues reduces CPD excision [63]. These considerations do not however exclude the possibility that site B may contribute to or even be required for less obvious DNA damage.

7. Where is XPC-RAD23B relative to TFIIH?

If XPD is scanning for lesions and site A is on the 3′ side of XPD, the lesion should start out on the 3′ side of XPD before scanning initiates. However, is this where XPC-RAD23B starts when it first recruits TFIIH? Before TFIIH, XPC-RAD23B, as the major protein initially detecting lesions, will have recognized the lesion by flipping out the undamaged DNA opposite to the lesion but will be primarily bound to the dsDNA 3 nts 3′ upstream from the lesion (Fig. 5B) [65,72]. XPC-RAD23B then recruits TFIIH to the damage site, but it is not known where the XPC-RAD23B-bound lesion is positioned relative to XPB and XPD. The pH domain in p62 that interacts with XPC is not modeled in any TFIIH models, and thus its position cannot decipher the relative XPC position. However, we postulate two possible locations. In model 1, XPC-RAD23B starts off on the 3′ side relative to XPD and as XPD scans for the lesion 5′–3′, the lesion must also be on the 3′ side relative to XPD (and XPB). Translocation by XPB would then open up the DNA 5′ to the lesion enabling concurrent ssDNA binding by XPD. In model 2, XPC-RAD23B starts off near the XPB and XPD interface. It incorporates the fact that as XPB is translocating, the dsDNA is moving 5′–3′ (based on XPB and the lesion-containing strand). A rough calculation based on the Pol II promoter opening indicates that XPB needs to translocate the same number of base pairs as the number of promoter nts that are opened up. By analogy to ~11 nts of ssDNA bound in the XPD/DinG structures, XPB would translocate ~11 base pairs. This movement would place the lesion, after XPB translocation, on the 3′ side of XPD. If the lesion started on the 3′ side as in model 1, then the XPB translocation would move the lesion even farther 3′ and XPD would need to make up for the gap.

8. What is the purpose of the [4Fe-4S] cluster in XPD?

XPD ranks among an elite group of replication and repair proteins that contain unique [4Fe-4S] cluster co-factors (including DNA polymerases, helicases, nucleases, primases, and glycosylases), as reviewed in [3]. There should be a selective advantage to having a [4Fe-4S] cluster, yet it is unclear what common advantage it might be other than structural integrity or in folding. There are many disadvantages. [4Fe-4S] clusters have multiple oxidation states, are sensitive to oxygen and cell stress, and are potentially toxic and mutagenic. They are metabolically expensive, requiring a specialized set of proteins for their assembly and incorporation in proteins. Why are electron-rich [4Fe-4S] clusters conserved in essential proteins that are so close to oxidation-sensitive DNA? One provocative hypothesis is that [4Fe-4S] clusters provide a signaling mechanism that uses charge transfer through duplex DNA. In the absence of mismatch or DNA damage that disrupts base stacking, electrons can travel over hundreds of base pairs [103–105]. Thus, effective charge transfer through the DNA would signal the absence of damage. Reduction of the [4Fe-4S] cluster in XPD reduces DNA binding [106,107]. Hence, if an electron is sent from a gap-filling DNA polymerase to XPD in TFIIH, which is only possible after creation of full duplex, it would serve as a signal that synthesis is completed and promote release from the DNA. Indeed, it is perhaps significant that lesion-scanning site A borders the [4Fe-4S] cluster and that one of the site A residues, Tyr192, bridges DNA and the [4Fe-4S] cluster. Although the DNA in this region is ssDNA, the bases are

stacked from the 3′ side, perhaps indicating directionality of the signal. Mutation of this residue abrogated the ability of TFIIH to scan for CPD lesions, which was attributed to widening site A [63]. It would be interesting to test if this mutation also interfered with the charge transfer between the cluster and DNA. Another provocative hypothesis is that the [4Fe-4S] aid in opening of the DNA, as this is a common function between polymerases, helicases, primase, and glycosylases. However, how [4Fe-4S] would do this is unclear.

9. The paradox of TFIIH in transcription initiation and NER

Evolution teaches us fundamental relationships about the cell biology of transcription initiation and repair. So, in thinking about TFIIH mechanisms, is it worth considering which of its roles came first? That TFIIH acts in both transcription initiation and NER seems paradoxical when viewed in terms of the differences in these processes, their distinct requirements, and the risks of combining their disparate activities. Why would TFIIH include a second SF2 helicase family enzyme XPD that is not required for transcription initiation and that adds an XPD [4Fe-4S] vulnerability to the transcription initiation process? Did a role for TFIIH in NER or transcription initiation come first? Transcription initiation is understood to be a more essential role. Or is it?

A key insight comes with the observation that the biggest block to replication (and thus to life) is transcription. The Merrikh lab used single molecule techniques to show in bacteria that only one of two potential replisomes was active in each cell at any one time during replication [108]. Only when transcription was inhibited were two active replisomes detected, suggesting that transcription caused the major block to replication. This dramatic blocking of the replisome was not due to DNA breaks, aberrant resolution of topological strain, or other DNA damages blocking the DNA polymerase per se, *i.e.* it is transcription that is the major block to replication. An important caveat to this argument is that TCR proteins are not essential. Given that this field is still making important discoveries, we speculate that removal of the RNA polymerase is so important, that the cell has developed multiple ways to compensate for loss of any one pathway.

If collision with transcription is the major replication block, then resolution of this collision between the RNA polymerase and the replication machinery must be the second most critical process after replication. As RNA polymerase stalled at a lesion and RNA polymerase that collided with the replication machinery have common logistical issues, could a version of TCR have been initially involved? Thus, transcription initiation and global DNA repair, albeit essential, could be a secondary evolutionary pressure on TFIIH. This sweeping line of thought may seem at first fanciful, overly simplistic, and impossible to prove, but it serves a purpose. When considering TFIIH mechanisms, it might be productive to consider TCR as the possible original process that has been subsequently adapted for transcription initiation and global NER. In other words, the answer to whether the chicken or the egg came first might be neither in the case of TFIIH. TC-NER may be central to truly understanding TFIIH, underscoring the value of further research on this possible progenitor pathway.

There are certain key observations consistent with the TC-NER progenitor idea. First, XPB translocase activity in TFIIH is not required for all promoter openings [48], suggesting that it is not essential per se and that TFIIH could have been added to improve efficiency. Second, the ~25 nt oligonucleotide released by XPF-ERCC1 and XPG in NER resembles the amount of DNA covered by an elongating Pol II (Fig. 8) [109]. Indeed, the relative lesion position in NER is similar [110]. In NER, the lesion is positioned in an ~25mer nt DNA bubble, with 5 nt of ssDNA 3′ to the lesion and ~20 nt 5′ to the lesion. In the case of a lesion at the Pol II active site, Pol II covers 7–8 nt on the 3′ side and 15 nt on the 5′ side. These numbers do not exactly match and must additionally take into account XPG and XPF-ERCC1 incision into the dsDNA, yet the similarity is striking. Third, the strand that would contain the NER-repaired lesion in the TFIIH-XPA-DNA complex has more

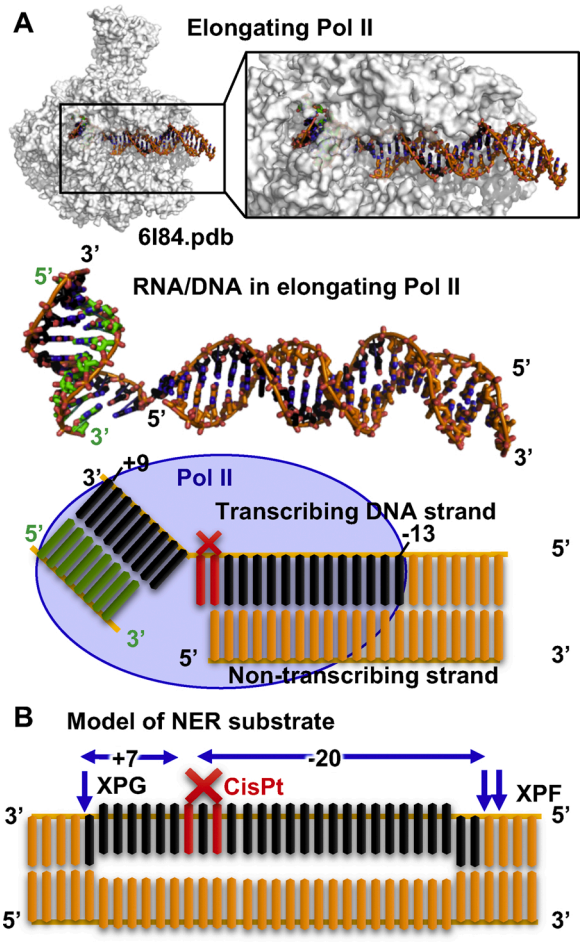

Fig. 8. DNA covered by Pol II resembles an NER incision product in size and positioning relative to a transcription-blocking lesion. (A) The DNA on the transcribing strand covered by an elongating Pol II. The top 2 views show yeast Pol II (surface representation) with RNA (green) and DNA (orange, black) or just the RNA-DNA (different orientations). The transcribing-strand region covered by Pol II is colored black. The bottom view is a schematic of the RNA/DNA. The cisPt position (red) is based on the Pol II structure with cisPt (2R7Z.pdb). (B) Schematic NER DNA bubble with a cisPt lesion. The oligonucleotide released in NER is marked in black. Figure adapted from Evans, 1997. The DNA bubble position is based on the relative dsDNA incision positioning of XPF and XPG.

interactions with XPB and is the same strand that is being transcribed and would block the polymerase if it contained a lesion (Fig. 2, black strand). Fourth, based on TFIIH-related diseases, Cockayne syndrome (CS), characterized by inability to resume RNA synthesis after DNA damage and, as such, defective TCR, has a far worse phenotype than xeroderma pigmentosum, caused by defective GG-NER.

Taking this model further, if TFIIH activity initially developed for TCR, then one would want to remove a trapped and replication-blocking Pol II from the DNA. If the dsDNA on either side of the trapped Pol II is cut, this would cause a dsDNA break, toxic or lethal at worse, mutagenic at best. It would be less toxic to make ssDNA breaks on either side. However, Pol II is clamped down on the dsDNA portions. If Pol II was altered such that it clamped on only one strand, removal of this one ss section would allow DNA polymerase to simply patch the removed section. To do this, it would be important to increase the elongating transcription bubble such that Pol II was covering only ssDNA and the blocking Pol II could be released, still clinging onto the damaged DNA strand (black in Fig. 8A schematic). Conversely, this hypothesis would explain why these lesions are removed as such large oligonucleotides during NER. How could one generate ssDNA the length of Pol II? The

answer is TFIIH.

How does TFIIH work during TCR? That is an open question, as TCR has never been reconstituted *in vitro*. An "Occam's razor" model is that Pol II is removed by ubiquitination and proteolysis [32,33], and TFIIH acts as it does in global NER. The pH domain of p62 that interacts with XPC also interacts with UVSSA in TC-NER, providing a possible unified mechanism between TC-NER and GG-NER for the engagement of TFIIH [36]. However, TFIIH is large enough such that XPB and XPD could access the DNA on either side of an elongating Pol II. This would require changes in XPB and XPD positioning, as well as changes in DNA compared to the Pol II PIC complex. Perhaps XPD is binding the ssDNA in an R-loop? Although proteins involved in TCR are known, their mechanism of action remains unknown. Nonetheless, such a model suggests an intriguing origin for NER.

10. What does the location of TFIIH disease mutations tell us about TFIIH functions?

Although the three major TFIIH diseases have discrete phenotypes, all are autosomal recessive and associated with sun sensitivity [111]. Xeroderma pigmentosum (XP) patients have 1000-fold increase in skin cancers; XP cells are defective for NER. XP/CS patients with mutations in NER proteins show severe neurological and developmental abnormalities, but with no increase in cancer risk. XP/CS cells are defective for both NER, TC-NER, and resumption of RNA synthesis after UV damage. In contrast to XP and CS, trichothiodystrophy (TTD) patients show symptoms of brittle hair and nails, intellectual impairment, decreased fertility, shorter life span and short stature with no increased risk of cancer [112].

Sequencing of TFIIH patient DNA and careful identification of disease missense mutations provide a powerful means to view the structure-function relationships of XPB, XPD, and p8. Unlike XPG disease mutations [100], each disease mutation in TFIIH cannot destroy the protein integrity or essential transcription initiation functions of TFIIH. Yet, there are serious disease phenotypes, it must disrupt one or more of the TFIIH functions. Notably disease-causing mutations center on XPD. There are 31 disease missense mutations in XPD, two in XPB, and one in p8 [113–125]. Four mutations have XP and TTD overlapping phenotypes, suggesting impact on multiple TFIIH functions. Understanding of how these mutants mis-function is informed by interpreting them in the context of TFIIH structures: indeed, the insights obtained have been increasingly informative as larger structures of complexes are determined [43,55,57].

Interestingly, most patient mutations map to secondary structure ends or loops, highlighting the significance of these positions. Whereas half the TTD mutations fall within helices, this is rare for XP and XP/CS mutations. Strikingly, 80 % of disease mutations localize to XPD helicase domains with none in transcription initiation-essential XPB translocase domains. Analysis of individual mutations can be found in our recently reported modeling and computational analysis of TFIIH [43].

In general, we find that disease mutations do not cluster by disease in the primary sequence or spatially in the structure (Fig. 9). However, they do fall into general structural categories. 1) The 13 TTD and 4 XP/TTD mutations map to protein-protein interfaces or interfacial helices in p8, XPB and XPD, suggesting that TTD mutations disrupt protein-protein interfaces (directly or through breaks in helices at interfaces). TTD mutations thus would weaken assembly of TFIIH subunits while retaining residual XPB translocase activity for essential transcription initiation function, consistent with the model that TTD partially disrupts TFIIH transcription initiation, resulting in segmental aging phenotypes.

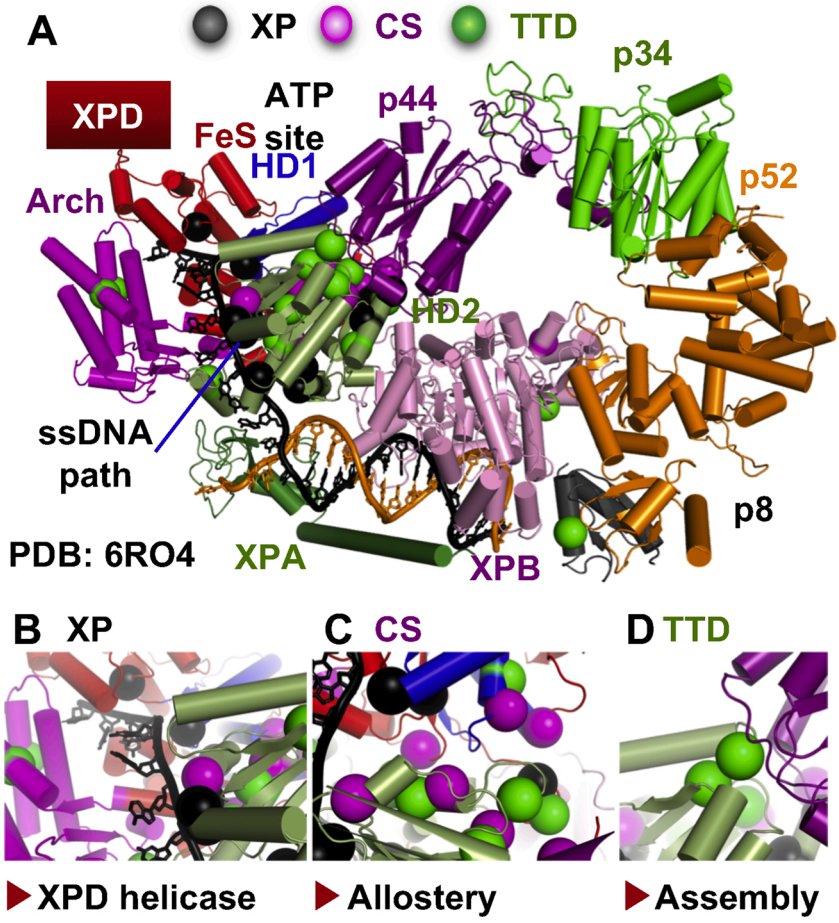

Fig. 9. TFIIH disease mutations mapped onto TFIIH-XPA-DNA. A) Positions of XP, CS, and TTD disease mutations (spheres) are shown on NER TFIIH-XPA-DNA structure. Each protein or subunit are colored as in Fig. 3, except XPD is colored by domain. Note that p62 was not modeled in this structure. B,C,D) Zooms highlight general features of each type of disease mutation. Most XP mutations are at XPD DNA or ATP-binding sites. Most CS mutations are at allosteric regions that change conformation. Most TTD mutations are at protein-protein interfaces. B and D are in same perspective as A. C is oriented to highlight HD1 (blue) and HD2 (moss green) interface. Parts of XPD were removed by depth slicing for clarity.

2) All 14 XP and XP/TTD mutations map to XPD. Surprisingly no XP mutations map to XPB, suggesting either a minor role in NER or it is difficult or impossible to separate XPB function in repair (*e.g.* ATPase) from that in transcription initiation. XP mutations tend to fall into positions near the ssDNA or ATP-binding regions of XPD, indicating a direct impact of XPD helicase-relevant functions. The remaining set of XP mutations map close to XPB, p44, and p62, suggesting that these interfaces are important for NER. 3) The seven XP/CS mutations in XPD or XPB map into regions implicated in allosteric communication between TFIIH dynamic communities [43]. We have defined communities as groups of residues that move together in our molecular dynamics analysis, analogous to a machine gear. Superseding the traditional separation of TFIIH by subunit, parts of a subunit polypeptide can belong to different communities, and one community can be composed of parts from multiple subunits. Thus, the location of XP/CS mutations in these communities point to allosteric changes in TFIIH as being critical for TCR. Interestingly, five of seven XP/CS mutations are close to p62, which spans communities and bridges the XPD ssDNA binding path, the XPD ATP binding site, and TFIIE. Although p62 has been difficult to model because of its molecular rigging-like structure, these XP/CS mutations support a critical role for p62.

11. How may TFIIH mechanisms impact cancer mutational signatures and biomarkers for therapy?

Mutational signatures are characteristic combinations of mutation types arising from specific mutagenesis processes such as defective NER. For precision cancer therapy, biomarkers can predict which patients will have the highest likelihood of responding to selected therapies such as DNA damaging agents or have adverse side effects with particular therapies as well as determine patient prognosis. TFIIH as a target for cancer therapeutics is under active investigation with inhibitor efforts targeting the kinase module or XPB (reviewed in [126]); however, there are as yet no TFIIH inhibitors in the clinic.

Is it possible to exploit genetic or functional repair defects in TFIIH through synthetic lethal mechanisms, as has been done for other DNA damage response proteins? Notably, Poly(ADP-ribose)ylation (PARylation) by PAR polymerase 1 (PARP1) and PARylation removal by poly (ADP-ribose) glycohydrolase (PARG) critically regulate DNA damage responses, so PARP1 inhibitors and PARG inhibitors are synthetic lethal with BRCA1, BRCA2, or other genes in the Fanconi anemia/BRCA pathway that cause defects in homologous recombination repair (HRR) [127,128]. In fact, PARylation and NER are linked at two stages. First, UV-DDB-associated PARP1 PARylates chromatin DNA and DDB2 at sites of UV damage [129]. Second, PARP1-mediated PARylation promotes XPC localization at DNA lesions, and XPA binds PAR chains [130–132]. Intriguingly, this link may extend to CS disease phenotypes: reduced NAD + levels, a result of excess PARylation that can cause cell death *via* the allosteric activation of Apoptosis-Inducing Factor [133], are associated with key CS phenotypes, and these phenotypes can be reduced in a mouse model system by NAD + supplementation [134,135].

In any case, these NER links to PARylation, where signatures and biomarkers were key to therapeutic success, highlight the question: can mutational signatures and biomarkers for NER be used as tools to guide precision therapies and clinical trials [136]? For example, ERCC2/XPD mutations in urothelial cancers show an increase in mutations attributed to COSMIC signature 5, a mutational signature characterized by a broad spectrum of base changes [137]. NER-deficient tissues also show increased Signature 8 mutations (increased double nucleotide substitutions), which may help predict NER-deficiency [138].

Importantly, mutational signatures are jointly shaped by DNA damage and DNA repair. NER prevents up to 99 % of missense mutations, almost uniformly across the mutation spectrum [139]. Investigating changes in UV mutation signature between adult skin tumors and those from XPC defective XP patients, showed a 30-fold increase in mutation rate per year in XP patients across all types of base substitutions. Yet, there was also a context dependent shift, with ~3 times more mutations acquired in NpCpT (N = A/G/T/C) and TpCpD contexts (D = A/G/T). As XPC deficiency inactivates GG-NER, the observed shift may reflect the loss of GG-NER efficiency despite compensation by TC-NER, which merits further study. Moreover, such NER defects may offer actionable knowledge for therapies and clinical trials. For example, a recent landmark study showed that XPD/ERCC2 helicase mutations that cause NER defects predict cisplatin efficacy for bladder cancer [140]. We therefore anticipate that the structural mechanisms considered here may shed light on mutations or polymorphisms likely to both cause repair defects and inform possible mutational signatures and biomarkers.

12. Perspective and unanswered questions

With its features and multi-functionality, TFIIH provides key insights and opportunities to learn about a quintessential macromolecular machine. By going beyond the molecular roles of TFIIH and its functional partners in DNA repair and transcription initiation, we examine how the composite proteins and the assembled TFIIH machine accomplish their activities. Building upon the field's current knowledge and ideas, we considered possible mechanisms for how XPB translocation leads to DNA opening through negative supercoiling; how XPA contributes to XPB unwinding with a helicase strand-separation pin; how ATP-binding, hydrolysis and release in XPD enables ssDNA movement and ssDNA loading, and how XPD uses sterically narrow channels to find and position lesions. We also examined insights learned from human disease mutations that currently teach us the fundamental biology that may be a necessary precursor to enable translational efforts to help these patents in the near term.

The detailed structural mechanisms that underlie TFIIH function are commensurate with the complexity of its functions in transcription initiation, NER, and TCR. Our analyses suggest that the continued combination of structures of TFIIH with different proteins, DNA substrates, and nucleotide states, biochemical experiments, cell biology, computational analyses and disease mutations will successfully tease apart the interwoven and step-wise coordination of these three processes. Notably, many questions remain including how TFIIH subunits are coordinated with each other and with partner proteins in NER, TC-NER, and transcription-initiation. For example, the combination of broad substrate specificity and excision accuracy in GG-NER involves concerted actions of TFIIH with XPC, RPA, XPA and XPG to shape the bubble and pre-incision complex, but how this works is enigmatic. Furthermore TFIIH acts in dsDNA break-induced transcription and may provide a mechanism of repair pathway choice [141], so how the TFIIH machine acts at the heart of different transcription and repair events merits attention.

Indeed, a key outstanding question is how does TFIIH control its nuclease activities? Incision by XPF-ERCC1 and XPG nucleases create the most toxic repair intermediate in NER suggesting they are both regulated and licensed, as is MRE11 nuclease for homology directed repair of dsDNA breaks [142]. Elegant Wood laboratory radiographs of NER DNA products show precise 26 nt excisions when the lesion is induced by Pt [143] suggesting incision is regulated and licensed when XPB and XPD have fully opened the NER bubble. Does such licensing and regulation come from a specific number of open nts, changes in the DNA conformation, blocked ATP hydrolysis or ATP/ADP exchange when a bulky lesion stuck at XPD site A or site B stalls XPD, how RPA is bound, or something else? Currently molecular dynamics analyses point to XPD at the center of TFIIH networked molecular communities, as best positioned to access the damage and mediate licensing of NER incisions [43].

In fact, we are struck by the occurrence once again of disrupted base unstacking in the DinG structures and predict that this will be a central mechanism for lesion sensing, as first proposed and observed for UvrB [144–146] and observed for uracil-DNA glycosylase lesion recognition and removal [147]. Moreover, the observed key roles of flexibility and

conformational change in NER suggest that experiments to define both flexibility and functional conformations in solution under near physiological conditions will be critically important to build upon the foundational cryo-EM structures, and we expect X-ray scattering will be a compelling method for such efforts [148]. Overall, we anticipate that the insights, ideas, and questions regarding TFIIH structure–activity relationships presented here may aid and focus ongoing experiments and research relating to both biology and medicine.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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