Transcription Pre-Initiation Complex with TFIIH Reveals Transcription-Ready, Repair-Regulated Helicase Machine from Combined Cryo-EM and Crystallography Datasets

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To begin transcription, Pol II depends on key general transcription factors (GTFs: TFIIA, TFIIB, TFIID, TFIIF, TFIIS, TFIIE and TFIIH) that recognize promoter DNA and assemble with the polymerase into a pre-initiation complex (PIC). Furthermore, transcription Factor IIH (TFIIH) plays critical and distinctly different helicase roles in transcription initiation and in Nucleotide Excision Repair. The helicase mechanisms for TFIIH activities in these two pathways are not understood, and published models of TFIIH are missing key parts of TFIIH subunits p62, p52, p44, p34, and XPB. We determined a largely complete TFIIH model based on advanced computational modeling plus integration of published cryoEM maps and crystal structures [1-6]. Strikingly, newly modeled regions interlace TFIIH subunits together like "molecular rigging" and are positioned for previously unexpected regulatory functions. The density maps identify key TFIIE/p62 interactions linking core-PIC to TFIIH. Furthermore, p62 caps the ATP binding site in XPD helicase subunit and both XPB and p62 are positioned against the ssDNA binding interface of XPD, indicating that the XPD repair functions are blocked in the apo TFIIH. The mostly complete TFIIH structure enabled molecular dynamics revealing which elements of TFIIH, independent of protein chain, move together as a community or act as "*the gears of the TFIIH machine*". We find that XPD is a dynamic anchor point and that promoter opening is linked to the global motions and dynamic networks within TFIIH. Notably, 31 of 34 single site patient mutations map to XPD and point to XPD as a central pivot point for TFIIH motions. Overall, the perplexing association of TFIIH with three different diseases is clarified by the finding that disease mutations cluster into distinct classes based on subunit assembly, XPD function, and networked communities.

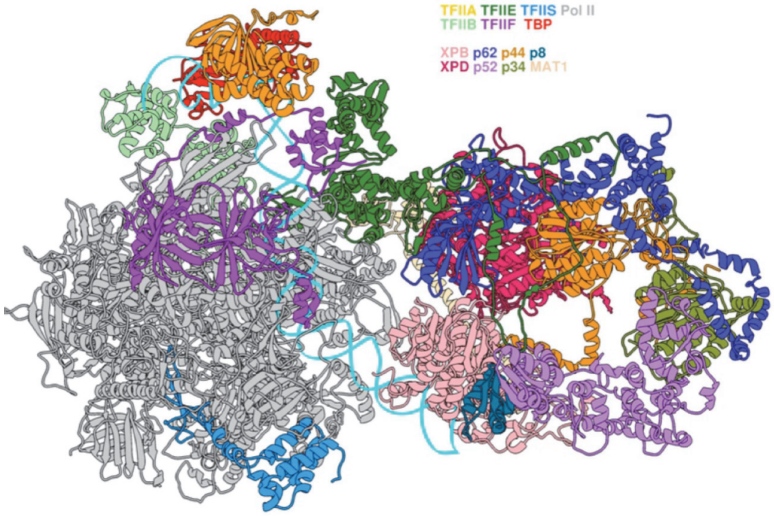

Fig. 1. PIC defined by Cryo-EM, crystallography, and computation.

References

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