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## Spotlight

## Take Your PIC

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**Cryo-electron microscopy has enabled unprecedented progress in the quest to reveal the structure of the whole transcription preinitiation complex. Four recent studies pave the way for a complete description of how transcription is initiated at near-atomic level.**

The efficient initiation of gene transcription by RNA polymerase II (Pol II) in response to activators requires the concerted activity of a ribosome-sized complex containing 76 polypeptides collectively known as the preinitiation complex (PIC). The PIC machinery (TFIIA, TFIIB, TFIID, TFIIIE, TFIIF, TFIIH, Pol II, and Mediator, MED) has been extensively studied at near-atomic resolution for >20 years [1]. However, information on the complete machinery has been lacking because the size and flexibility of TFIID and MED have long represented major challenges for its structural characterization. Importantly, in all previous PIC structural studies [1], TATA-binding protein (TBP) was substituted for TFIID, which did not reflect truly physiologically relevant PIC complexes, and it remained unclear how TFIID supported PIC assembly, especially on TATA-less core promoters.

Recently, the laboratories of He [2] and Cramer [3] succeeded in solving the structure of human MED-PIC<sup>TBP</sup> complex, and the laboratory of Xu was able to determine not only the structure of human PIC<sup>TFIID</sup> complexes on different core promoters [4] but also the structure of the whole MED-PIC<sup>TFIID</sup> [5]. Because most previous structural data came from yeast models [1], an important advance of these breakthroughs is that they reveal the cryo-

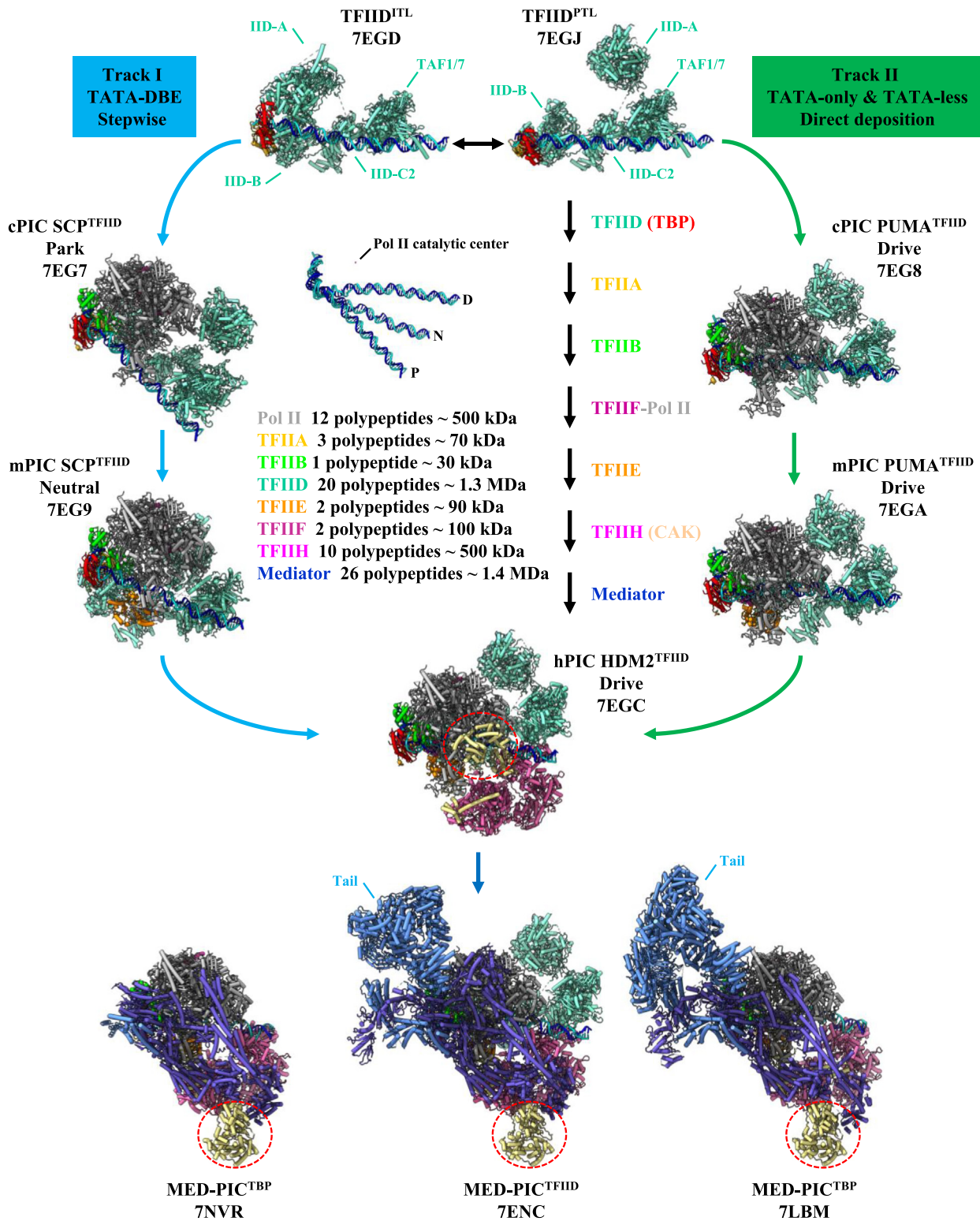
electron microscopy (cryo-EM) structure of the mammalian PIC containing MED and TFIID at an overall resolution of ~4Å (Figure 1). They not only confirm the remarkable conservation of the transcription initiation machinery in eukaryotes [6] but also shed light on differences likely driven by the presence of metazoan-specific MED subunits and its much larger Tail module in human MED-PIC complexes.

The cryo-EM structures of human TFIID previously reported by the laboratory of Nogales [7] were a remarkable achievement that also raised important questions regarding its assembly and dynamics. In this context, the PIC<sup>TFIID</sup> structures determined by Chen and colleagues [4] in distinct assembly states are a real *tour de force*. The authors determined the structures of 25 different intermediate complexes on 13 different promoters representing three configurations [TATA-only, TATA-less, and TATA-DBE (TFIID-binding element that includes Initiator, MTE, and DPE elements) core promoters]. These structures provide a wealth of structural insights that help to explain how TFIID could accommodate diverse core promoters. The authors proposed a convincing PIC assembly model (direct and stepwise promoter deposition) that involves extensive conformational changes driven by the arrangement of promoter elements (Figure 1). PIC assembly starts with a shared TFIID-TFIIA conformation that first diverges into two distinct promoter-dependent tracks (track I TATA-DBE, and track II TATA-only and TATA-less) that finally converge at the same fully assembled holo-PIC.

With the cryo-EM structure of PIC<sup>TFIID</sup> at hand, the last challenge was to solve the structure of the complete PIC, and the laboratory of Xu has done it masterfully [5]. 3D classification revealed four distinct MED-PIC<sup>TFIID</sup> conformational states, confirming that MED is particularly dynamic and flexible during PIC assembly [2,3,6,8]. MED sits at the top of the complex, covering the whole

PIC, and is thus able to act as a pendulum. In its equilibrium position, all PIC components are positioned to properly orient and load the Pol II C-terminal domain (CTD) into the TFIIH kinase CDK7 active site. The Xu [5], He [2], and Cramer [3] MED-PIC structures adopt a very similar overall architecture. Major differences reside in the presence of the complete MED Tail module and in partial CTD heptapeptide repeats densities in the structures determined by the Xu [5] and He [2] laboratories, suggesting that the Tail may modulate MED-CTD interactions. Importantly, the Tail points towards the upstream promoter DNA, consistent with its function as an interaction interface for numerous transcription factors [9]. Further, although cooperativity between MED and TFIID has been reported, the MED-PIC<sup>TFIID</sup> structure indicates that MED and TFIID occupy opposite sides of Pol II and do not directly interact with each other [5]. One should note that the PIC complexes were assembled on strong core promoters that are capable of engaging in high-affinity interactions with the PIC. The models built from cryo-EM data therefore only represent the most stable conformation. Various PIC assembly pathways may coexist *in vivo* depending on the composition of the promoter structures.

One remarkable difference between MED-PIC and PIC<sup>TFIID</sup> is the location of the TFIIH CDK7/cyclin H kinase complex (CAK) that occupies very distinct positions in the PIC depending of the presence of MED. Weak densities of CAK were present in previous cryo-EM reconstructions, but its position and orientation could not be clearly established at that time [6]. In the PIC<sup>TFIID</sup> structure [4], CAK is positioned next to the Pol II Rbp4/Rbp7 stalk in close proximity to the C-terminal end of Rbp1. By contrast, in the MED-PIC complexes [2,3,5], CAK is located separately from the rest of the PIC at the tip of the hook domain of the MED Middle module (Figure 1). The flexible nature of the MAT1 linker that physically bridges CAK to the TFIIH core likely supports



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(See figure legend at the bottom of the next page.)

these vastly different CAK positions. It was also shown that TFIID and MED can both stimulate phosphorylation of the CTD by CAK [3,4]. However, the location and the relative levels of phosphorylation along the CTD remain largely unknown. Recent results [10] suggest that the proximal half of human CTD composed primarily of the repeating consensus heptad YSPTSPS supports RNA synthesis and cotranscriptional processes, whereas the distal half of the CTD that consists primarily of divergent motifs is required for rapid transcription activation. Distinguishing TFIID- versus MED-specific CAK-mediated CTD phosphorylation effects is an important question for future research and will be key to a better understanding of the functional differences that may exist between the two halves of the CTD.

Technological innovations in cryo-EM and (high-resolution) crystal structures of individual PIC subunits or submodules determined over the past two decades by many groups [1,9] have led to this

outstanding achievement. The fascinating reports by Abdella, Chen, Rengachari, and colleagues provide an exciting framework for addressing important remaining questions such as how exactly the exchange of the numerous factors involved in the different transcriptional stages (initiation, reinitiation, pausing, and elongation) that compete for the same binding surfaces on Pol II is regulated, what is the molecular basis of the mutually exclusive binding of Pol II and CDK8 MED kinase module (CKM) to MED, and how phosphorylated CTD leads to dissociation of MED from Pol II.

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#### Declaration of Interests

The authors declare no conflicts of interest.

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**Figure 1. Transcription Preinitiation Complex (PIC) Assembly.** All views are shown in a similar orientation superimposed with RNA polymerase II (Pol II). (Inner section) Color scheme for protein components of the PIC, numbers of each component proteins, and PIC sequential assembly are indicated. (Top) TFIID is divided into IID-A (with a dissociable TBP), IID-B, and IID-C (TAF1/7 + IID-C2) modules as indicated. Promoter-bound TFIID-TFIIA exists in two topological arrangements (ITL, initial TBP-loading in which the TBP-containing IID-A module is loaded onto upstream promoter; and PTL, post TBP-loading in which the IID-A module binds to the IID-C module) and serves as an entry state for initial PIC assembly that first diverges into track I (left) and track II (right) that converge at the same fully assembled holo-PIC (hPIC<sup>TFIID</sup>). The position of the Pol II catalytic center relative to the park (P), neutral (N), and drive (D) promoter conformations are indicated; adapted from [4]. PIC assembly on track I (left, blue arrows) occurs on strong promoter-positioning element (TATA box and DBE) that defines the promoter trajectory in the park conformation. PIC assembly on track II (right, green arrows) occurs on TATA-only and TATA-less promoters that leads to the drive conformation. The CAK (in khaki highlighted with red circles) occupies very distinct positions in hPIC<sup>TFIID</sup> [4] compared to the Mediator MED–PIC<sup>TBP</sup> [2,3] and MED–PIC<sup>TFIID</sup> [5] complexes (bottom). MED Tail (cornflower blue) is indicated. PDB IDs are listed for each structure. Abbreviations: cPIC, core PIC; DBE, TFIID-binding element; HDM2, TATA box promoter derived from human HDM2 gene; hPIC, holo-PIC; MED, Mediator; mPIC, intermediate PIC; PUMA, TATA-less promoter derived from human PUMA gene; SCP, super promoter.