Minireview

Srinivasan Rengachari*, Sandra Schilbach and Patrick Cramer*

Mediator structure and function in transcription initiation

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Abstract: Recent advances in cryo-electron microscopy have led to multiple structures of Mediator in complex with the RNA polymerase II (Pol II) transcription initiation machinery. As a result we now hold in hands near-complete structures of both yeast and human Mediator complexes and have a better understanding of their interactions with the Pol II pre-initiation complex (PIC). Herein, we provide a summary of recent achievements and discuss their implications for future studies of Mediator and its role in gene regulation.

Keywords: coactivator; gene regulation; mediator; RNA polymerase II; transcription.

1 Introduction

The evolutionarily conserved Mediator complex is a quintessential part of the eukaryotic RNA polymerase (Pol) II initiation apparatus (Allen and Taatjes 2015; Conaway and Conaway 2011; Kornberg 2005) and plays an important role in the transcription of protein-coding genes under both basal and activator-driven conditions (Kim et al. 1994; Lacombe et al. 2013; Takagi and Kornberg 2006). Mediator consists of four structural modules called the head, middle, tail and kinase modules (Allen and Taatjes 2015). The Pol II associated part of Mediator comprises 21 subunits in yeast and 26 in humans (Tsai et al. 2014). Of these, 15-subunits form the functional core that encompasses the head and middle modules (Liu et al. 2001) which are conserved throughout eukaryotes (Bourbon 2008). The Mediator kinase module is incompatible with Pol II binding (Tsai et al. 2013) and is formed by four subunits which are also conserved (Bourbon 2008).

At the time of our last review of Mediator (Plaschka et al. 2016), several research groups had employed integrated structural biology methods to arrive at an atomic model for the Mediator head module (Lariviere et al. 2012; Robinson et al. 2012) and a topological model for the middle module (Lariviere et al. 2013; Robinson et al. 2015), which led to a composite model for core Mediator (cMed) (Lariviere et al. 2012, 2013). The structures showed that the Mediator head module consists of eight submodules – the shoulder, arm, spine, joint, moveable jaw, finger, tooth and nose (Figure 1a) (Lariviere et al. 2012). Low resolution cryoEM studies revealed the overall architecture of the tail module (Tsai et al. 2014), the dissociable Cdk8 kinase module (Tsai et al. 2013) and Mediator containing Pol II initiation complexes (Plaschka et al. 2015) thus defining how mediator locates to Pol II (Plaschka et al. 2015). In this review, we summarize the latest developments in the structural characterization of Mediator since 2016, and outline future perspectives.

2 Mediator structure

To improve our structural understanding of cMed, a cryo-EM study conducted with an endogenous preparation of *Schizosaccharomyces pombe* (Sp) Mediator reported a nominal resolution of 4.4 Å (Tsai et al. 2017). However, due to a lack of high resolution information, the subunit assignment in the resulting model was limited to the subunits Med1 and Med14 of cMed, while the backbone model of cMed was extended including a partial model of Med27. A second study determined the structure of recombinantly purified Sp cMed by X-ray crystallography at 3.4 Å resolution (Nozawa et al. 2017). The higher resolution allowed unambiguous assignment and visualization of 15 of the 16 subunits of cMed, with Med1 being absent in the electron density. In this structure, details of the Mediator middle module and its association with the

Current address: Odyssey Therapeutics GmbH, Industriepark Höchst G875, Brüningstraße 50, D-65929 Frankfurt am Main, Germany.

***Corresponding authors: Srinivasan Rengachari and Patrick Cramer**, Department of Molecular Biology, Max Planck Institute for Multidisciplinary Sciences, Am Fassberg 11, D-37077 Göttingen, Germany,
E-mail: srinivasan.rengachari@mpinat.mpg.de (S. Rengachari), patrick.cramer@mpinat.mpg.de (P. Cramer). https://orcid.org/0000-0003-4237-8258 (S. Rengachari)
Sandra Schilbach, Department of Molecular Biology, Max Planck Institute for Multidisciplinary Sciences, Am Fassberg 11, D-37077 Göttingen, Germany

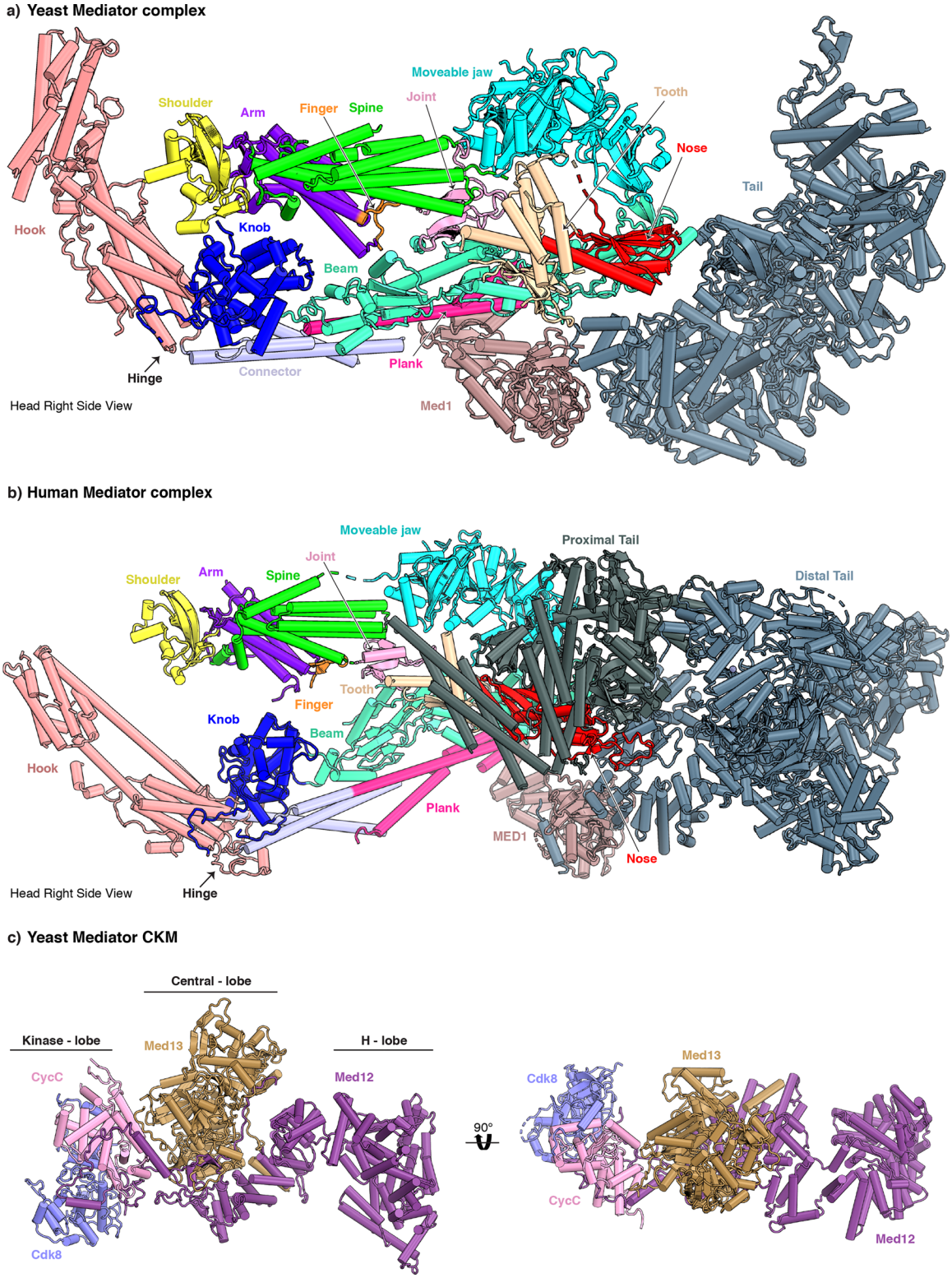

Figure 1: Overall structure of the eukaryotic Mediator complex. (a) The yeast Mediator complex is represented as a cartoon, highlighting its sub-modules within the head, middle and tail modules. The model was generated by compositing the high resolution structures of each module (PDB codes: 7JMN, 7UIO – tail module; 8CEN – head, middle modules) (Gorbea Colon et al. 2023; Schilbach et al. 2023; Zhang et al. 2021). (b) Cartoon representation of the human Mediator complex (PDB code: 7EMF) (Chen et al. 2021) with its sub-modules indicated by colour. The colour code is consistent with panel (a). (c) Structure of the Sc Mediator CKM in two views, represented as a cartoon (PDB code: 7KPX) (Li et al. 2021). Subunits are distinguished by colour and respective sub-modules are indicated with labels.

Mediator head module were observed. The middle module contains five distinct sub-modules named the beam, plank, hook, knob, and connector (Figure 1a) (Nozawa et al. 2017). Binding of the middle module does not induce any structural changes in the 7-subunit head module (Lariviere et al. 2012). Using the structure of Sp cMed, the *Saccharomyces cerevisiae* (Sc) Mediator model was also extended (Nozawa et al. 2017). The obtained model showed how *in vivo* mutations of head and middle module subunits are located at their interaction surfaces and disrupt yeast Mediator complex integrity (Nozawa et al. 2017). Though these two studies significantly advanced our understanding of cMed, the structure of the tail module of Mediator remained unknown.

The structure of the tail module was addressed in a study of Mediator from the thermophilic yeast *Chaetomium thermophilum* (Ct) (Zhang et al. 2021). The cryo-EM structure of endogenously isolated Ct Mediator was determined in complex with Pol II (Zhang et al. 2021). Though all of the Mediator head, middle and tail modules were well resolved, the way Mediator associated with Pol II remained poorly defined due to flexibility. The overall architecture of Ct Mediator was consistent with prior work describing low-resolution Sc Mediator cryo-EM reconstructions (Tsai et al. 2014). The tail module of Ct Mediator only contains three (Med5, Med15 and Med16) of the five tail subunits characterized in yeast and lacks Med2 and Med3. However, Ct Med15 (1625 aa) is substantially larger than its Sc ortholog (1081aa) and forms an ensemble with Med2 and Med3. The tail module is stabilized through the central role of Med16 in bridging Med5 and Med15 and is connected to the rest of Mediator through an interaction of Med15 with the C-terminus of Med14 (Figure 1a). This study helped to explain how Med14 forms a central 'beam' which connects all three modules of Mediator and contributes to the stability of the complex (Cevher et al. 2014). In addition, the structured parts of Med1 showed how this subunit links the Mediator middle and tail modules (Figure 1a).

The structure of the mammalian Mediator complex was determined by four research groups using cryo-EM, from two different species – mouse (Zhao et al. 2021) and humans (Abdella et al. 2021; Chen et al. 2021; Rengachari et al. 2021). Preparation of mammalian Mediator was achieved through endogenous or recombinant isolation methods. In agreement with older cryo-EM data (Tsai et al. 2014), the overall structure of the mammalian Mediator resembles its yeast counterpart, in particular the head and middle modules (Figure 1b) (Tsai et al. 2014). Four out of five additional metazoan-specific subunits of Mediator (MED27, MED28, MED29, MED30) anchor the tail module to cMed, mostly through contacts with the head module, as inferred from previous biochemical studies (Figure 1b) (Tsai et al. 2014). This part of the Mediator complex is referred to as the 'proximal tail' (Rengachari et al. 2021). The fifth subunit MED26 associates with the middle module as reported previously (Tsai et al. 2014), with only its C-terminal region presenting a structured fold that interacts with the connector. The other half of the tail module constituted by MED15, MED16, MED23, MED24 and MED25 forms a horse shoe like structure with extensive inter-subunit interactions (Figure 1b). This part is called the 'distal tail', indicating its position from the Mediator core. The structure of MED23 as a part of the full Mediator ensemble contains multiple heat-repeats and is consistent with its crystal structure reported in isolation (Monte et al. 2018). All four reported structures converge to a unified model for the mammalian Mediator which helps us to understand the molecular details of Mediator mutations in various diseases. The MED17 L371P mutation, which causes infantile cerebral and cerebellar atrophy (Kaufmann et al. 2010), is located in the tooth region of the head module and may interfere with formation of the secondary structure in this domain, leading to Mediator instability. Similarly, the MED23 R617Q mutation, which causes non-syndromic autosomal recessive intellectual disability (Hashimoto et al. 2011), disrupts an interaction network at the core of this subunit (Monte et al. 2018; Prajapati et al. 2006).

Another critical component of the Mediator complex is its dissociable Cdk8 kinase module (CKM). In a recent structural study, the cryo-EM structure of the Sc Mediator CKM was determined (Li et al. 2021). The overall structure resembles an asymmetric M-shape, placing the Cdk8/CycC (human CDK8/CycC) containing Kinase-lobe at one end and the Med12-containing H-lobe on the other end (Figure 1c) (Li et al. 2021). The two lobes are connected by the Central-lobe formed by Med13. The cryo-EM map with a nominal resolution of 4.4 Å enabled the unambiguous localization of subunits Med12 and Med13 within the CKM (Tsai et al. 2013). The structure reveals that the N′- and C′–terminal regions of Med12 contain flexible linkers interspersed with helices that extend like tentacles to anchor CycC and Med13. The N-terminal region of Med12 stabilizes the T-loop of the enzymatic component of Cdk8, thereby promoting its activation (Klatt et al. 2020). The authors used this structure also to extend the atomic model for the Mediator-CKM complex using previously published low resolution cryo-EM reconstructions (Tsai et al. 2013). In addition, a biochemical study using highly purified reconstitutions showed how the CKM directly competes with Pol II for Mediator binding (Osman et al. 2021). Using 4tU-seq the authors also explained how the CKM plays a dual role in Mediator regulation (Osman et al. 2021). CKM, under normal conditions represses gene expression by restraining Mediator to the upstream

activating sequences (UAS); but in response to stress, the Cdk8 activity may release the Mediator complex to prime for transcription initiation (Osman et al. 2021).

Although these structures go a long way in expanding our understanding of the Mediator complex, many of the Mediator subunits (16 in yeast, 19 in humans) contain extended stretches of intrinsically disordered regions (IDRs), which were not observed in these structures (Toth-Petroczy et al. 2008). For example, the subunit MED15 is highly disordered (83 % in yeast, 78 % in humans) (Xue et al. 2010) and only a small stretch of its sequence has so far been resolved in any of the Mediator structures (Abdella et al. 2021; Chen et al. 2021; Gorbea Colon et al. 2023; Zhao et al. 2021). The IDRs are also present in Pol II, general transcription factors such as TFIID, and transcriptional activators. Recent work from several groups identified a central role for these IDRs in forming transcriptional condensates through liquid-liquid phase separation (Boehning et al. 2018; Boija et al. 2018; Cho et al. 2018; Sabari et al. 2018). Mediator exhibits an extensive tendency to form and co-localize to such condensates (Jaeger et al. 2020; Zamudio et al. 2019). Live cell imaging in yeast showed that Mediator is spatio-temporally constrained from diffusion, reinforcing the functional role for its coalescence within the condensates (Nguyen et al. 2021). This has been reviewed elsewhere (Richter et al. 2022).

3 Mediator interactions and functions

To understand Mediator function, structures of its complexes with Pol II and the pre-initiation complex (PIC) are required. Such studies were incepted by two groups, demonstrating the reconstitution of the Mediator containing Sc PIC and its structural characterization by cryo-EM. Whereas one study assembled a 52-subunit complex including the tail module of Mediator, the resolution of the obtained map was limited to a range of 15–18 Å (Robinson et al. 2016). In the second study, the 46 subunit PIC lacked the tail module of Mediator but reported a resolution range of 4.5–7 Å (Schilbach et al. 2017). Both studies confirmed the position of Mediator on the PIC, as reported previously (Plaschka et al. 2015) and were in agreement with respect to the location of the CDK-activating kinase (CAK) module of TFIIH between the shoulder and hook of the Mediator head and middle modules, respectively. Complemented by cross-linking data and previously determined crystal structures of the Mediator head module in complex with an Rpb1 CTD heptapeptide repeat (YSPTSPS), both studies derive the putative trajectory of the Rpb1 CTD towards the active site of the TFIIH kinase Kin28 (human CDK7) (Robinson et al. 2012). The study presenting the higher resolution structure also embossed the previously reported Mediator interactions with Pol II and the general transcription factor and PIC component, TFIIB (Plaschka et al. 2015). The efforts to obtain the atomic structure of the yeast PIC-Mediator complex culminated in a cryo-EM structure of the cMed containing complex on a nucleosomal template at a nominal resolution of 3.0 Å (Figure 2a) (Schilbach et al. 2023). This study reported a local resolution of 3.3 Å for both the head and middle modules of cMed. In spite of the overall high resolution reported in this work, the TFIIH CAK remained at low resolution in the periphery of the complex, indicating its flexibility in yeast. Nontheless, the study permitted the visualization of eleven out of the 26 CTD repeats of Pol II subunit Rpb1. The repeats were observed as three different fragments engaging in extensive interactions with nine of the 15 subunits of cMed (Figure 2a) (Schilbach et al. 2023). The observed CTD repeats within this structure helped to put many years of functional work on the role of the CTD and the minimum requirement of eleven CTD repeats for yeast viablility in a molecular perspective (Babokhov et al. 2018; Sawicka et al. 2021; Kim et al. 1994; Nonet et al. 1987; West and Corden 1995).

Of the four studies which reported the structure of the mammalian Mediator, three described the structure of human Mediator in complex with the entire Pol II PIC containing TFIIA, TFIIB, TBP/TFIID, TFIIE, TFIIF, TFIIH and promoter DNA (Figure 2) (Abdella et al. 2021; Chen et al. 2021; Rengachari et al. 2021). The overall resolution of the structures in these studies were in the 4–4.5 Å range (Abdella et al. 2021; Chen et al. 2021; Rengachari et al. 2021), with the local resolution of Mediator modules extending as high as to 2.8 Å (Abdella et al. 2021; Chen et al. 2021; Rengachari et al. 2021). Similar to the yeast Mediator, the human orthologue interacts with the PIC by binding the Pol II stalk and dock domains and TFIIB through its head module (Figure 2b). The human Mediator complex additionally interacts with the E-ribbon of TFIIE (Figure 2). Importantly, the high resolution of these structures enabled the unambiguous modelling of the TFIIH CAK and its interaction with the Mediator complex. The CAK is positioned between the shoulder (MED6) and the hook (MED14-N terminal region, MED19) domains of Mediator, with the interaction limited to the CDK7 kinase subunit. Two of the three studies also reported fragments of the RPB1 CTD at the CDK7 kinase active site and within the Mediator complex (Figure 2) (Abdella et al. 2021; Chen et al. 2021). In the structure with the highest resolution (Chen et al. 2021), two distinct fragments of the CTD were observed that amounted to ~ 5 heptapeptide repeats (Figure 2b). The structure of human Mediator-containing Pol II PIC complexes solves an important puzzle in how Mediator

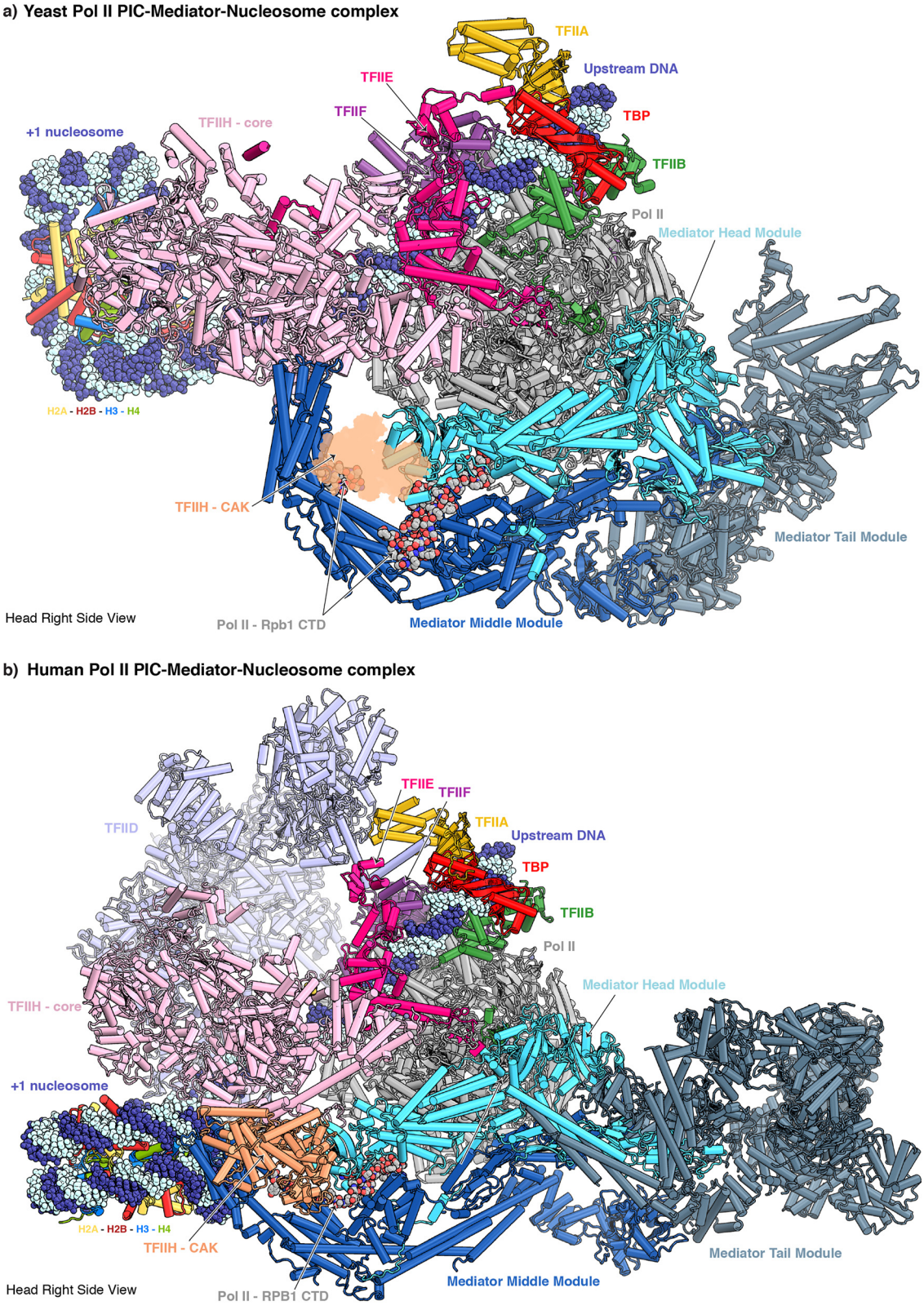

Figure 2: Structure of the eukaryotic Pol II PIC-Mediator-nucleosome complex. (a) Cartoon representation of the yeast Pol II PIC-Mediator-nucleosome complex structure. The structure of yeast Mediator was derived from a composite model as described in Figure 1a (PDB codes: 7JMN, 7UIO, 8CEO) (Gorbea Colon et al. 2023; Schilbach et al. 2023; Zhang et al. 2021). The sub-modules of the major components are distinguished by colour. The position of TFIIH-CAK as observed in cryo-EM map EMD-16611 is indicated as a translucent orange surface. (b) The structure of human Pol II PIC-Mediator-nucleosome complex in cartoon representation (PDB code: 8GXQ) (Chen et al. 2022). The colour code is the same as in panel (a).

stimulates basal transcription. The stabilization of CAK by Mediator through direct binding promotes a ~ 4-fold increase in the phosphorylation of RPB1 CTD which leads to increased transcriptional output under basal conditions (Chen et al. 2021; Rengachari et al. 2021). A very recent study determined the cryo-EM structure of human Mediator containing Pol II PIC on a nucleosomal DNA template. In addition to the previously identified interactions with Pol II, TFIIB, TFIIE and TFIIH CAK, this structure revealed that the hook region of the Mediator middle module contacts the +1 nucleosome (Figure 2) (Chen et al. 2022). Whereas the resolution in this region was low, the authors used other atomic structures to identify positively charged patches within the hook relevant to this interaction. They further used mutational analysis to identify the subunits MED19 and MED26 as the functional players regulating this interaction and also show that these mutations reduce the transcription activity of Pol II *in vitro* (Figure 2b). Interestingly, MED19 and MED26 have also been shown to be targeted by transcription factors to silence neuronal gene expression (Ding et al. 2009).

Mediator interactions with the Pol II initiation machinery involve intricate dynamics. In the Mediator-containing Pol II PIC structure in yeast, the Mediator middle module rearranges upon binding to the PIC through swinging of the hook and knob, stretching of the beam and rotation of connector and plank regions (Abdella et al. 2021; Chen et al. 2021; Rengachari et al. 2021; Schilbach et al. 2017; Zhao et al. 2021). Whereas the human Mediator also adopts these changes, the binding of RPB1 CTD induces further rearrangements in the head module (Chen et al. 2021). The tail module of free Mediator was observed to alternate between bent and extended conformations (Chen et al. 2021). Both of these conformations have also been captured in structural studies of PIC-bound Mediator (Abdella et al. 2021; Chen et al. 2021). Additionally, the binding of human Mediator to Pol II induces a movement of the Pol II stalk formed by subunits RPB4 and RPB7. This rearrangement repositions the clamp-bound transcription factor TFIIE in closer contact to Mediator (Aibara et al. 2021; Grohmann et al. 2011; Rengachari et al. 2021).

4 Conservation of mediator structure

The structure of Mediator has now been determined at high resolution using five different eukaryotic species spanning yeast, mouse and humans. In addition to its basic composition, also the overall structure of the protein complex is highly conserved across evolution, both in its free state as well as in a functionally associated form with the Pol II PIC (Abdella et al. 2021; Chen et al. 2021; Rengachari et al. 2021; Robinson et al. 2016; Schilbach et al. 2017; Tsai et al. 2014; Zhao et al. 2021). However, a closer comparison of the structures reveals that the mammalian Mediator complex undergoes local structural changes to accommodate the additional metazoan-specific subunits. In particular, the nose and the tooth sub-modules of the head module rotate to integrate the proximal tail subunits. The hook, shoulder, connector and plank regions of the middle module also adopt different orientations. Likewise, the central beam formed by MED14 shows minor deviations in its trajectory which leads to a tighter interaction between the Mediator spine and Pol II stalk in humans. The apparently more stable association between Mediator and TFIIH CAK in humans as compared to yeast may also be a product of these minor evolutionary changes. The biggest structural divergence between yeast and mammalian Mediator is the conformation of the tail module. In comparison to yeast, the distal tail subunits of mammalian Mediator contain ordered protein regions and the proximal tail region stably connects it to the Mediator core (Figure 1a, b). This results in an overall rigid tail conformation that could be structurally well resolved (Abdella et al. 2021; Chen et al. 2021; Zhang et al. 2021; Zhao et al. 2021). Another functionally important aspect of Mediator conservation is its highly similar binding to the RPB1 CTD heptapeptide repeats. The high resolution structures of yeast and human Pol II PIC–Mediator complexes show that the CTD extends across the cradle formed by the concave surface of Mediator and binds between the head and middle modules, thereby stabilizing Mediator conformation within the PIC (Abdella et al. 2021; Chen et al. 2021; Schilbach et al. 2023).

5 Outlook

In recent years, the collective work of several groups using a combination of biochemical and structural methods has improved our understanding of the structure-function relationship in eukaryotic Mediator-containing complexes. These studies highlight the high degree of conservation in Mediator structure from yeast to humans and show how the additional subunits present in metazoans integrate within the complex and induce local structural changes. The structures of Mediator in complex with Pol II-containing complexes of varying composition explain how Mediator directly interacts with the PIC and the +1 nucleosome and promotes transcription initiation.

The wealth of information from these works provides a basis for further studies directed towards the structure of

Mediator, its complexes with other components of the transcription apparatus and the functional implications thereof. In particular, the interplay between Mediator and transcriptional activators is of great interest to the community. How do the plethora of transcriptional activators and regulators that have been identified to interact with various parts of Mediator influence its dynamics and function in transcription initiation (Malik and Roeder 2010; Quevedo et al. 2019)? As a first step, a structural model of an activator dependent dimerization of Mediator-bound Pol II PIC on a divergent promoter has emerged (Gorbea Colon et al. 2023). Also, provided that transcriptionally active promoters are surrounded by well-positioned, regularly spaced nucleosomes (Mavrich et al. 2008; Valouev et al. 2011), how does the Mediator-containing PIC interact with chromatin beyond the +1 nucleosome? Another unresolved question is the structure of Mediator in complex with its CDK8 kinase module. This structure will be important to characterize the regulation of Mediator in response to stress signals and define the role of CDK8 in influencing multicellularity and development processes in metazoans as well as its contribution to human diseases (Allen and Taatjes 2015; Yin and Wang 2014; Osman et al. 2021). Answering these questions will require additional efforts to resolve very large and flexible protein-nucleic acid complexes and will shape our understanding of the role of the general transcriptional co-activator Mediator in the future.

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