# Transcription regulation by the Mediator complex

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Abstract | Alterations in the regulation of gene expression are frequently associated with developmental diseases or cancer. Transcription activation is a key phenomenon in the regulation of gene expression. In all eukaryotes, mediator of RNA polymerase II transcription (Mediator), a large complex with modular organization, is generally required for transcription by RNA polymerase II, and it regulates various steps of this process. The main function of Mediator is to transduce signals from the transcription activators bound to enhancer regions to the transcription machinery, which is assembled at promoters as the preinitiation complex (PIC) to control transcription initiation. Recent functional studies of Mediator with the use of structural biology approaches and functional genomics have revealed new insights into Mediator activity and its regulation during transcription initiation, including how Mediator is recruited to transcription regulatory regions and how it interacts and cooperates with PIC components to assist in PIC assembly. Novel roles of Mediator in the control of gene expression have also been revealed by showing its connection to the nuclear pore and linking Mediator to the regulation of gene positioning in the nuclear space. Clear links between Mediator subunits and disease have also encouraged studies to explore targeting of this complex as a potential therapeutic approach in cancer and fungal infections.

Specific transcription factors
Also known as sequence-specific DNA-binding factors (which serve as activators or repressors) that bind to specific DNA sequences in regulatory regions and are essential for regulated transcription.

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The expression of genetic information of a cell starts with transcription. This fundamental, conserved and extremely complex biological process is tightly regulated to ensure that genetic programmes are adapted to cell requirements. Transcription deregulation leads to serious diseases, including cancers.

In eukaryotes, all protein-coding genes are transcribed by RNA polymerase II (Pol II). Regulated transcription by Pol II requires the concerted activity of various transcription activators and repressors (collectively known as specific transcription factors), which bind to their target sequences in regulatory regions of transcription to modulate — either by direct protein–protein interactions or through effects on chromatin (remodelling of structure, modification of histones, etc.) — the assembly and activity of the transcription machinery. These functions of transcription factors are further modulated by transcription co-regulators (FIG. 1).

Mediator of RNA polymerase II transcription (Mediator) was identified more than 20 years ago as a co-regulator of transcription by genetic and biochemical approaches in yeast and mammalian cells (reviewed in REFS 1–3). Since then, it has been established that Mediator is an evolutionarily conserved multisubunit protein complex (comprising 25 subunits in budding yeast and up to 30 subunits in humans (BOX 1)) that is generally required for transcription — it serves as a functional bridge between transcription factors and basal transcriptional machinery, including Pol II and general transcription factors (GTFs; including transcription initiation factor IIA (TFIIA), TFIIB, TFIID, TFIIE, TFIIF and TFIIH), promoting the assembly of the preinitiation complex (PIC) on core promoters[4–6]. Individual deletion of 10 of the 25 yeast Mediator subunits (Med4, Med6, Med7, Med8, Med10, Med11, Med14, Med17, Med21 and Med22) is lethal, and unperturbed Mediator function is required for expression of nearly all protein-coding genes in yeast[4–6]. In all reported cases, knockout of various mammalian Mediator subunits was embryonic lethal in accordance with a widespread requirement for Mediator in the activation of transcription[7–11] (see also a discussion on the generality of Mediator action in REF. 12). Taking TATA-elements (TATA-box or TATA-like elements[13]) as an example, PIC assembly involves the recruitment of TATA-box-binding protein (TBP) — a component of TFIID — to the core promoter region. Mediator is independently recruited to enhancer regions via its direct interactions with transcription factors bound to these regions (reviewed in REF. 14); following chromatin looping, it establishes interactions with PIC components, contributing to recruitment and/or stabilization of these components and promoting PIC assembly[5,15–21] (FIG. 1).

Co-regulators
Multisubunit protein complexes (which serve as co-activators or co-repressors) that interact with transcription factors and participate in transcription regulation. This group includes complexes that directly influence the basal assembly of transcriptional machinery as well as chromatin modifiers or remodellers that act on a chromatin structure.

General transcription factors
(GTFs). Proteins or protein complexes that are essential for promoter recognition by RNA polymerase II (Pol II), its function and recruitment. This class of proteins were identified as essential components for basal transcription from a specific promoter from *in vitro* Pol II transcription assays.

Mediator also stimulates phosphorylation of the Pol II carboxy-terminal domain (CTD) by cyclin-dependent kinase 7 (CDK7; also known as serine/threonine-protein kinase Kin28 in yeast, a component of TFIIH), which triggers Pol II release from promoters, thereby allowing transition from transcription initiation to productive elongation[22]. In addition, on the basis of *in vitro* data, a subset of GTFs and Mediator were proposed to constitute a scaffold that remains on promoters and facilitates transcription reinitiation[23]. However, *in vivo* evidence for such reinitiation events and their mechanisms is lacking. It should also be noted that Mediator has been implicated in other steps of transcription after PIC formation and Pol II promoter release (reviewed in REFS 12,14,24). However, these aspects are not discussed in detail in this Review.

Since the discovery of Mediator, many studies have investigated its fundamental role in transcription regulation. However, mechanistic studies of Mediator, in particular, in the native *in vivo* context, are hampered by the large compositional complexity, the conformational flexibility and the recently unveiled compositional dynamics of this complex — properties that can also explain the implication of Mediator in a number of human pathologies (BOX 2). This Review focuses on recent advances in understanding the molecular mechanisms of Mediator in transcription activation, mainly with the employment of structural biology and functional genomics. This work, which has been produced by many laboratories, reveals new aspects of Mediator activity and regulation and provides interesting perspectives for future studies as well as for the development of therapeutics targeting Mediator in the context of disease. Mediator has been extensively studied in yeast models; however, relevant discussion below also covers new insights into Mediator in metazoans. In particular, regulation of Mediator recruitment and the concomitant changes in module composition as well as the interplay between Mediator and the PIC are discussed. Novel findings connecting Mediator to the nuclear pore that extend Mediator function from gene expression regulation to gene positioning in the nuclear space are also covered. Finally, a discussion of therapeutic approaches to target Mediator in the context of diseases, including cancer and fungal infections, is provided.

## Mediator composition and structure

As a large multisubunit complex, Mediator is particularly challenging for structural studies, and the entire structure is typically elusive for classical crystallography. However, Mediator subunits form stable subcomplexes, and the entire structure of the complex can be divided

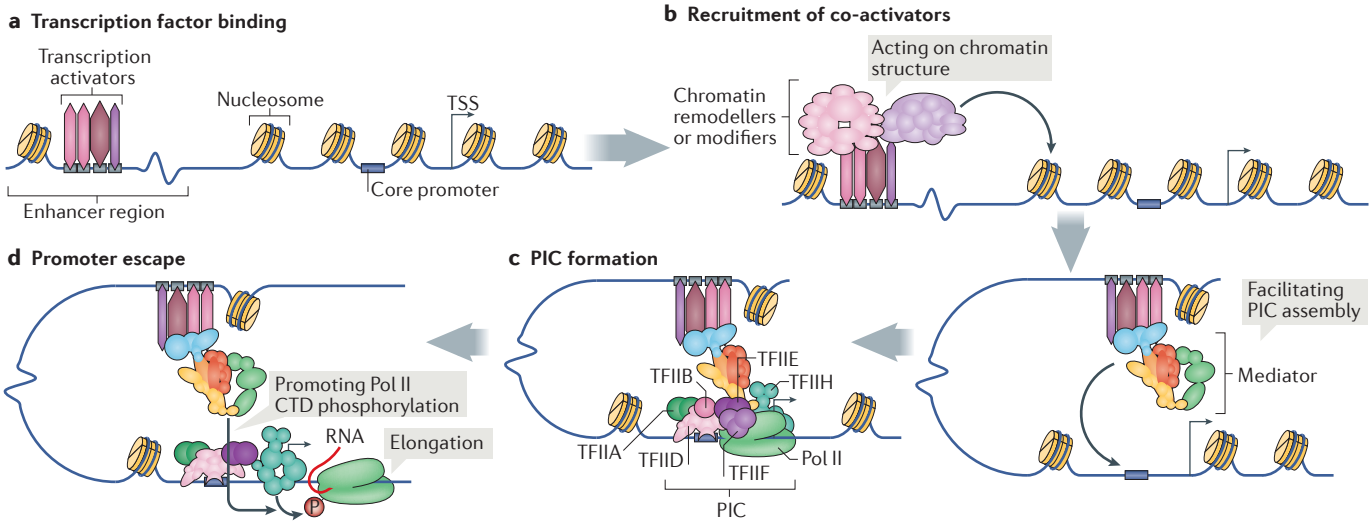


Figure 1 | **Transcription activation by RNA polymerase II.** A simplified model for the main steps of transcription initiation by RNA polymerase II (Pol II) in a chromatin context. Of note, the size and proportions of the depicted components do not reflect their actual dimensions. **a** | Transcription activation starts with the binding of transcription factors (in this case, activators) on enhancer regions (enhancers in metazoans or upstream activating sequences in yeast). These enhancer regions are located at different distances (hundreds of bases apart in yeast and sometimes megabases apart in metazoans) from the core promoters. The transcription start site (TSS) is indicated by an arrow. **b** | Activators then recruit co-activator complexes that act as chromatin modifiers or remodellers to alter chromatin structure and to make it more accessible for other factors. Other co-activators are then recruited that act directly on the assembly of basal transcriptional machinery, the so-called preinitiation complex (PIC). Many co-activators act in cooperation, and some have functions both as chromatin regulators and as co-activators, contributing to PIC formation. The functions of chromatin regulators are not depicted in detail. In general, transcriptional co-regulators transmit the regulatory signals from the specific transcription factors to the PIC. Mediator of RNA polymerase II transcription (Mediator) is one of the key co-activator complexes. **c** | The PIC is assembled at the core promoter. It includes Pol II (12 subunits), general transcription factors: transcription initiation factor IIA (TFIIA; 2 subunits), TFIIB, TFIID (comprising TATA-box-binding protein (TBP) and 14 TBP-associated factors), TFIIE (2 subunits), TFIIF (2–3 subunits) and TFIIH (10 subunits, including 7 core subunits and a 3-subunit kinase module (TFIIK) containing cyclin-dependent kinase 7 (CDK7; also known as serine/threonine-protein kinase Kin28 in yeast)). Multiple steps and pathways could be involved in PIC assembly *in vivo*, and Mediator acts to facilitate recruitment and/or stability of different PIC components. **d** | CDK7 (yeast Kin28) phosphorylates (P) the carboxy-terminal domain (CTD) of the largest Pol II subunit at Ser5, which is necessary for Pol II to escape from the promoter and for the transition from the initiation step to the elongation step. This phosphorylation is also regulated by Mediator.

Box 1 | **Evolution and conservation of the Mediator complex**

The mediator of RNA polymerase II transcription (Mediator) complex was discovered in budding yeast through genetic and biochemical studies[6,22,80,123] and has been identified in mammals, flies, other eukaryotes[25] and, more recently, plants[25,124]. Different names were initially used for Mediator subunits, and a unified nomenclature has now been proposed[125].

In evolution, Mediator emerged in eukaryotes and is conserved from unicellular eukaryotes to metazoans[25]. The primary sequences of Mediator subunits are divergent in different organisms, with limited identity or similarity for some subunits, but structural homologues of all yeast Mediator subunits are present in humans[14,25]. On the basis of multiple sequence alignments and secondary structure features, a set of conserved Mediator subunits was identified in most eukaryotes. The presence of predicted intrinsically disordered regions (IDRs) in many Mediator subunits in metazoans, plants and fungi might partially explain the divergence of Mediator primary sequences — even though the sequence conservation is relatively weak for some Mediator subunits, the location of IDRs is very similar, suggesting general structural conservation. It might also reflect the important role of these features for Mediator flexibility, both conformational and regulatory, and its capacity for multiple protein–protein interactions[126,127] or for phase separation, which is an emerging mechanism for controlling the activity of proteins and their complexes, for example, in enhancer-based and super enhancer-based transcription regulation[128,129].

Mediator composition depends on the organism, and the overall number of Mediator subunits increased during eukaryotic evolution. Accordingly, several human Mediator subunits, including Mediator subunit 23 (MED23), MED25, MED26, MED28 and MED30, are absent from the yeast complex. In addition, three of four subunits of the vertebrate CDK8 kinase module: cyclin-dependent kinase 8 (CDK8), MED12 and MED13 have paralogues: CDK19, Mediator subunit 12-like protein (MED12L) and Mediator subunit 13-like protein (MED13L), respectively, which assemble in a mutually exclusive manner. The presence of homologous structural folding for several Mediator heterodimers, including Med7–Med21, Med11–Med22 and Med4–Med9 in yeast, suggests that gene duplications and diversifications, also proposed for other large protein complexes, have occurred during the rapid evolution of the Mediator complex to increase its regulatory capacity[130–132].

into clearly distinct modules[25–28], which has greatly facilitated structural analysis of Mediator. These include the head, middle, tail and CDK8 kinase modules; the last of which is transiently associated with the rest of the complex[26] (FIG. 2). Structural and biochemical data indicate that — in both yeast and humans — the head and middle modules constitute the Mediator essential core, which is necessary for transcription regulation, whereas the tail and CDK8 kinase modules serve regulatory functions[29,30]. This modular organization of Mediator probably reflects different functionalities of Mediator components (see discussion below).

High-resolution crystallographic models have been reported for several yeast Mediator subunits, submodules[31] and the head module[32–34]. The crystal structure of the 15-subunit Mediator core structure, which comprises the head and middle modules in fission yeast and is the largest Mediator subcomplex characterized at high resolution to date, was recently reported[35]. In addition to the structure of the Mediator head module, this work provided high-resolution structural details for the Mediator middle module[35]. Recent structural studies on the Mediator complex based on essential improvements in electron microscopy (EM) methodology combined with mass spectrometry and molecular modelling approaches have provided important advances in understanding the architecture of yeast Mediator[27,28,30,32–34,36–38] (Supplementary information S1 (table)). Most structural studies of the Mediator complex have been performed in yeast. Less detailed information has been obtained so far for human Mediator, but conserved Mediator architecture has now been demonstrated in humans[27,29]. Indeed, the overall Mediator architecture visualized by EM is conserved between the yeast and human complexes[27,39].

Recently, the overall architecture of the yeast Mediator complex has been substantially revised using EM approaches[27,28]. These studies provided a complete molecular description of the spatial organization of Mediator and precise interactions between the three main modules, the head, middle and tail modules. In line with the proposed spatial organization of Mediator[27,28] (FIG. 2), the 3D model of budding yeast Mediator (lacking the Cdk8 kinase module) revealed close intermodular interactions and a key importance of Mediator subunit 14 (Med14), which makes contact with all three main Mediator modules in Mediator organization[36]. This critical role of the MED14 subunit for Mediator architecture and function was also demonstrated in studies with human, *in vitro*-reconstituted Mediator core complexes[29] and further confirmed by high-resolution cryo-EM structure analysis of the 16 core subunits of yeast Mediator[38]. Thus, it can be concluded that MED14 functions as a central backbone that connects the Mediator head, middle and tail modules.

A detailed view of Mediator in the presence of the CDK8 kinase module remains to be determined. Using EM and biochemical approaches, the subunit organization, overall structure and interactions of Mediator with the CDK8 kinase module were analysed in budding yeast and in humans[40,41]. These studies suggested that the Mediator–CDK8 kinase module interaction is largely conserved in eukaryotes and proposed that the CDK8 kinase module interferes with the early steps of PIC assembly.

Taken together, recent structural analyses of the Mediator complex constitute a major step forward in building a high-resolution structural model for the entire Mediator complex. Understanding of its structure is in turn essential to discern the mode of action of Mediator and the precise impact of conformational changes within the complex, which depend on the composition of Mediator and the contacts it makes with nuclear proteins to regulate transcription. Recent insights into these functional aspects of Mediator activity are discussed below.

## Recruitment to regulatory regions

To regulate transcription, Mediator is recruited to enhancers in metazoans and upstream activating sequences (UASs) in yeast (referred to here as enhancer regions); this occurs via direct interactions with specific transcription factors (reviewed in REF. 14). Then, Mediator contacts the PIC components at respective promoters, which can be located at various distances from the enhancer regions (hundreds of bases in yeast and up to many megabases in metazoans). These events constitute essential steps in the function of Mediator in transcription activation, but the molecular details and the regulation of Mediator recruitment to the regulatory regions of chromatin are only now starting to emerge.

Preinitiation complex
(PIC). A large protein complex that assembles on core promoters to position the RNA polymerase. The main components of the PIC of RNA polymerase II (Pol II) are six general transcription factors, Pol II and Mediator.

Core promoters
Minimal DNA sequences close to the transcription start site (TSS) that are sufficient to direct accurate transcription initiation. TATA-elements represent one of the core-promoter elements.

TATA-elements
Consensus sequences (such as TATA-boxes) or sequences presenting some mismatches from the consensus (such as TATA-like elements) in promoters that are bound by TATA-box binding protein.

Pol II carboxy-terminal domain
(CTD) A domain of the largest RNA polymerase II (Pol II) subunit that is important for various steps of the transcription cycle and RNA processing and contains multiple repeats of the heptapeptide sequence YSPTSPS, which are subject to phosphorylation (at Ser2, Ser5 and Ser7) and other post-translational modifications.

***Regulation of Mediator–activator interactions.*** Mediator is able to contact hundreds of transcription activators via its different subunits and participates in the transmission of regulatory signals under the control of these specific transcription factors[14]. These Mediator–transcription factor interactions frequently involve Mediator tail module subunits, but they are not restricted to this module. Mediator was proposed to integrate signals from different transcription factors (reviewed in REFS 12,24), and changes in Mediator complex conformation induced by transcription factor binding were suggested to contribute to transcription regulation (reviewed in REF. 14). Nevertheless, it remains to be determined how Mediator is able to participate in transcription regulation that is controlled by hundreds or even thousands of different transcription factors and how exactly the regulatory signals are transmitted from transcription factors to Mediator. Likewise, little is known about which protein–protein interactions determine recognition and binding of Mediator to the various transcription factors and how these contacts are regulated to modulate Mediator recruitment and function.

Post-translational modifications (PTMs) of Mediator subunits or activators, including phosphorylation by specific kinases, might represent key aspects of this regulation, which has been proposed in yeast and mammals[42–48]. Taking phosphorylation as an example, yeast Med13 was shown to be a substrate of protein kinase A, and this modification modulates Mediator activity[42]. Phosphorylation of Med2 in the Mediator tail module by the Cdk8 kinase module has a specific effect on iron-regulated transcriptional activator Aft1-dependent transcription in yeast[48]. More generally, an *in vivo* proteomic analysis of yeast Mediator revealed multiple phosphorylation sites (localized to 17 of 25 subunits), and the phosphorylation levels of these sites change during stress[47]. In particular, phosphorylation of Med15 in the Mediator tail module prevented stress-induced transcription during normal conditions, potentially by suppressing Mediator association with activators involved in expression of stress-induced genes[47]. In human cells, oestrogen receptor (ER)-dependent transcription is an example of a complex mechanism of transcription regulation, which involves phosphorylation of ER and several co-regulators, including Mediator, by DNA-dependent protein kinases[44]. As another example, in mouse cells, phosphorylation has been shown to control the interaction of Mediator with transcription activator ETS domain-containing protein ELK1 and, in consequence, to control transcription activation by ELK1 (REF. 49). ELK1 can be phosphorylated by ERK on multiple sites within its transcription activation domain, but with varying kinetics for the different sites. Notably, phosphorylation of sites that show fast kinetics and intermediate kinetics of modification was shown to promote Mediator–ELK1 interaction and transcription activation, whereas phosphorylation of sites that demonstrate slow kinetics of modification inhibited Mediator recruitment and transcription. This work proposes an interesting mechanism for fine-tuning Mediator–activator interactions to shape the transcriptional response that could potentially apply to other activation systems. However, in many cases, the mechanistic understanding of the role of PTMs on Mediator–transcription factor interactions and on Mediator functions remains to be elucidated.

***Specific modules and module-composition changes drive Mediator recruitment.*** To assist in PIC assembly, activator-recruited Mediator needs to be delivered to the forming PIC at the core promoters. Thus, Mediator is expected to interact with the PIC directly at core promoters. However, chromatin association of Mediator was detected predominantly on the enhancer regions in both yeast cells and mammalian cells, with only low signals at core promoters[50–58]. Recently, inactivation or depletion of Kin28 (which phosphorylates the CTD of Pol II to regulate promoter escape) allowed Mediator stabilization on the core promoters, providing evidence that, as expected, Mediator does associate with the PIC on core promoters *in vivo*[59,60]. Even when stabilized at core promoters, Mediator was shown to co-associate with enhancers, supporting the mechanism whereby Mediator is first recruited to enhancer regions and subsequently contacts promoters[61].

Studies using a Kin28-deficient model were extended to investigate the interactions of Mediator with chromatin regulatory regions in more detail. It has been shown that the head and middle modules of Mediator are central for the recruitment of the complex to core promoters (and for the interaction with Pol II and for PIC assembly), whereas activator–Mediator interactions

Box 2 | **Mediator subunits in human diseases**

As expected given their essential role in transcription activation, genetic variations or changes in expression level that affect human mediator of RNA polymerase II transcription (Mediator) subunits lead to a number of pathologies, thus emphasizing the importance of studies of Mediator function for human health[110,111]. For example, mutations in Mediator subunit 12 (MED12) were found in X-linked mental retardation syndromes[133,134]. A mutation in MED17 was associated with infantile cerebral atrophy[135], and a mutation in MED23 co-segregated with intellectual disability[136].

Because cancers often arise as a consequence of dysfunction in gene expression, it is not surprising that aberrant Mediator function is also implicated in a number of cancers[110,111]. Potential mechanisms have been proposed for Mediator dysfunction in some cancers. For example, the gene encoding cyclin-dependent kinase 8 (CDK8) is a colorectal cancer oncogene, and its presence promotes the expression of genes controlled by β-catenin and β-catenin-dependent transformation[114]. MED29 regulates key cellular functions in pancreatic cancer with both oncogenic and tumour-suppressive functions[137]. Moreover, Mediator subunits have been proposed as therapeutic targets for cancer. For example, MED1 and MED17 are overexpressed in 50% of prostate cancers, and lowering their levels in cancer cells inhibits proliferation, slows the cell cycle and induces apoptosis[138]. Similarly, lentivirus-mediated silencing of MED19 has been shown as a strategy to inhibit the cell growth and proliferation of breast cancer cells[139]. Furthermore, CDK8 inhibition has been shown to be a potential strategy to boost the expression of tumour suppressors[112]. Because Mediator was found to interact physically and functionally with a number of virally encoded proteins, including those of human pathogenic viruses, targeting Mediator–viral protein contacts could be relevant to human diseases[140–146]. It has also been shown that targeting Mediator–transcription factor interfaces can be used as a strategy to treat drug-resistant fungal infections[113].

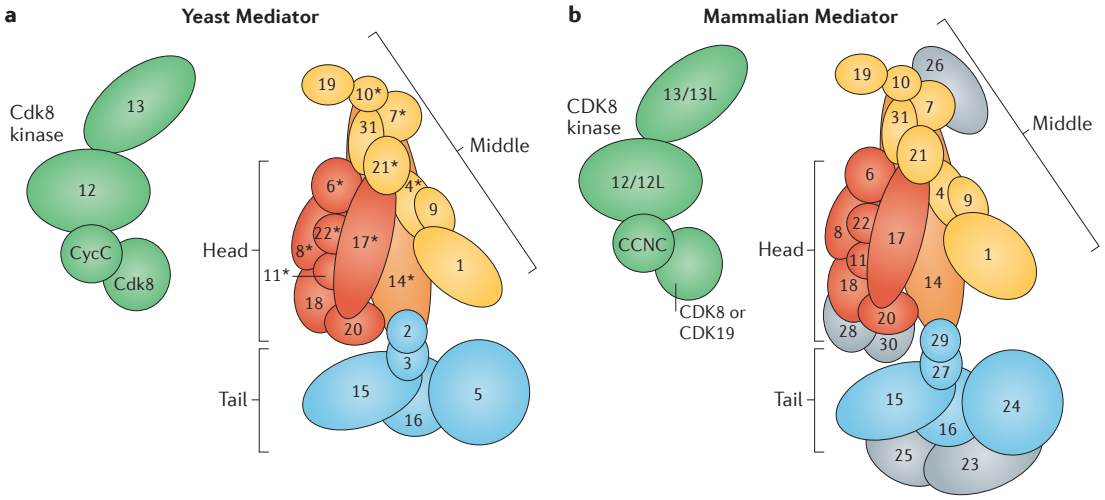


Figure 2 | **Subunit composition of the Mediator complex.** Schematics representing the modular organization of the budding yeast mediator of RNA polymerase II transcription (Mediator) complex (part **a**) and the mammalian Mediator complex (part **b**) based on a recently revised architecture derived from electron microscopy data[27,36]. Mediator comprises four distinct modules: a head module (in red), middle module (in yellow) and tail module (in blue), which function as the main modules and the CDK8 kinase module (in green), which is transiently associated with the complex. Mediator subunit 14 (MED14), which links all three main modules (head, middle and tail), is indicated in orange. Many Mediator subunits form multiple contacts within the complex, and the schematics represent the relative location of the Mediator subunits. Individual subunits are depicted by their subunit number, except for cyclin-dependent kinase 8 (CDK8), CDK19 and cyclin-C (CCNC; also known as RNA polymerase II holoenzyme cyclin-like subunit (CycC) in yeast[125]). The 10 Mediator subunits (of the 25 total) that are essential for viability in budding yeast are indicated by asterisks. In metazoan Mediator, MED24, MED27 and MED29 are orthologous to Med5, Med3 and Med2 in yeast, respectively. CDK8, MED12 and MED13 (components of the CDK8 kinase module) also have paralogues (CDK19, Mediator subunit 12-like protein (MED12L) and Mediator subunit 13-like protein (MED13L), respectively) in vertebrates. The exact module localization of five metazoan-specific subunits (MED23, MED25, MED26, MED28 and MED30) remains to be assigned. These subunits are thus indicated in grey. Potential assignment of MED30, MED26 and MED23 to the head, middle and tail modules, respectively, has been proposed. For example, MED23 was shown to be a part of the tail submodule with MED16 and MED24 (REFS 7,9). The figure was adapted with permission from REF. 147, Elsevier.

occur preferentially through the tail module. Notably, Mediator containing all subunits was recruited to the enhancer regions, but the Cdk8 kinase module was absent from the complex when it was associated with core promoters, and Mediator interaction with the PIC was only transient[62,63] (FIG. 3). This dynamic and transient nature of Mediator interactions with the core promoters and with the PIC components potentially contributes to its function in transcription regulation by accommodating multiple contacts while avoiding incompatibilities or clashes.

These data provide *in vivo* evidence for differential functionalities of Mediator modules and may explain the emergence of this modular structure in eukaryotic evolution. It should be noted that the precise sequence of events from loss of the Cdk8 kinase module to formation of the PIC and Mediator–PIC interactions remains to be defined. In addition, as the head and middle modules constitute the functionally essential core of the Mediator complex, whereas the tail and Cdk8 kinase modules are regulatory[29,30], it remains to be investigated exactly how Mediator contacts activators via its tail and how other modules contribute to this interaction to regulate transcription. In this regard, the use of DNA templates, in which enhancer regions and core promoters are clearly separated from each other (unlike in the currently used templates, where these regions are close together), in *in vitro* analysis will help to address these issues.

Together, these studies in yeast revealed that separate modules govern the interactions of Mediator with enhancer regions and with core promoters and that the transition to a promoter-bound complex is associated with changes in its module composition (loss of the Cdk8 kinase module). They also indicate that association of Mediator with the PIC is dynamic and transient. Mechanisms of module-composition changes may also be relevant for mammalian Mediator. First, preferential association of metazoan Mediator with enhancers and moderately low core-promoter binding was observed in murine embryonic stem (mES) cells, in different types of differentiated cells and in human myeloma cells, similar to that observed in yeast cells[52,57,58]. The involvement of mammalian Mediator in promoting chromatin looping between enhancers and core promoters was also suggested, which implies that Mediator is able to contact both enhancers and promoters[52,64,65] (see discussion below). Furthermore, dissociation of the CDK8 kinase module of Mediator during transcription activation was shown in human cells[66,67]. Nevertheless, the presence of several metazoan-specific Mediator subunits and the implication of metazoan Mediator in

Activating RNAs
Long non-coding RNAs with enhancer-like function that interact with Mediator and activate their neighbouring genes.

mechanisms occurring after Pol II recruitment (reviewed in REFS 12,14) suggest that a greater complexity of mechanisms is involved in Mediator recruitment, activation and function in metazoans than in yeast. For example, metazoan Mediator has been proposed to be involved in regulation of promoter proximal pausing of Pol II, in addition to its role in transcription initiation[12,14].

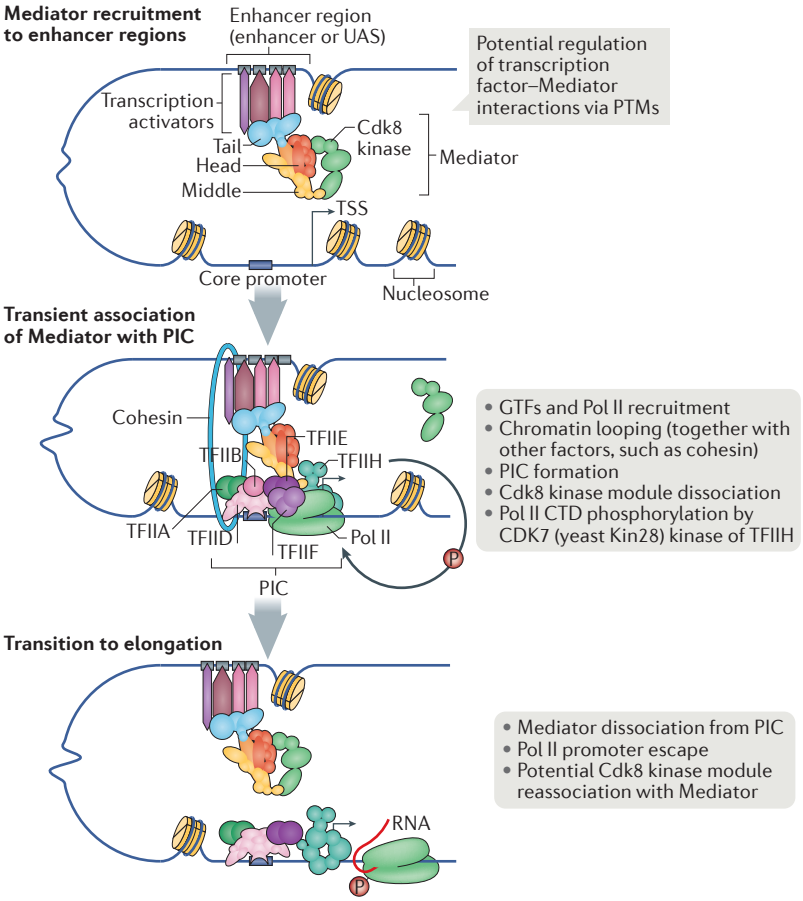


Figure 3 | **Mediator recruitment and transient association with the core promoter.** Mediator of RNA polymerase II transcription (Mediator) does not directly bind DNA but interacts with chromatin through intermediates: transcription factors at enhancer regions and preinitiation complex (PIC) components at core promoters. These interactions established between different regions of chromatin are associated with chromatin looping, which brings transcription activators into the vicinity of the forming PIC. Mediator can cooperate with other nuclear components in chromatin looping, for example, with cohesin, as has been shown in mouse embryonic stem cells[52]. Mediator associates preferentially with the enhancer regions, and its occupancy at core promoters is generally low. The Mediator complex containing all four modules (head, middle, tail and Cdk8 kinase) is recruited to enhancer regions through Mediator–transcription factor interactions. The involvement of the tail module is particularly evident in these contacts, and these interactions might be further modulated by post-translational modifications (PTMs)[49]. Mediator then transiently contacts general transcription factors (GTFs) and RNA polymerase II (Pol II) that are recruited to the core promoter for PIC formation. This transition is associated with dissociation of the Cdk8 kinase module from Mediator. It remains to be determined in which precise sequence the loss of the Cdk8 kinase module, the PIC formation and Mediator–PIC interactions occur. It has been proposed that the essential function of Mediator during PIC assembly occurs through a transient association of this complex with PIC components. It is possible that once the Mediator complex has dissociated from PIC, it is re-bound by the Cdk8 kinase module, but this remains to be formally confirmed. CDK7, cyclin-dependent kinase 7 (also known as serine/threonine-protein kinase Kin28 in yeast); CTD, carboxy-terminal domain; P, phosphorylation; TFII, transcription initiation factor II; TSS, transcription start site; UAS, upstream activating sequence.

***Bridging of enhancer regions and promoters by Mediator.*** As discussed above, Mediator is recruited by transcription factors bound to enhancer regions and contributes to PIC assembly on core promoters that could be located at a considerable distance from enhancers, particularly in metazoans. In our current understanding, Mediator functions as a physical bridge between transcription factors bound to enhancer regions and the PIC at core promoters, which suggests that chromatin looping is an important event in transcription regulation by Mediator. A model involving chromatin looping is supported by studies in yeast (discussed above), which demonstrated that a single Mediator complex is bound to both enhancers (UASs) and core promoters, thereby providing direct *in vivo* evidence for the function of Mediator as a dynamic physical link between these DNA elements[63]. In metazoans, chromatin looping by Mediator was suggested to be involved in nuclear receptor-dependent activation of gene expression[68,69].

Mediator might cooperate with other nuclear components for chromatin looping. Accordingly, a functional connection between Mediator and cohesin complexes that allows for DNA looping between enhancers and core promoters has been demonstrated in mES cells[52]. Mediator was shown to interact with cohesin, which can form rings to connect two DNA regions, thereby connecting the enhancers and core promoters of active mES genes[52]. Cooperation between Mediator and non-coding RNAs (activating RNAs and enhancer RNAs) in the chromatin looping that occurs between enhancers and core promoters was also suggested[64,65]. Further elucidation of mechanisms governing enhancer–promoter looping and the dynamics and role of Mediator in this process will be key to expanding our understanding of transcription regulation.

## Mediator in PIC assembly

The most recognized function of Mediator in transcription regulation is in stimulation of PIC formation[70,71]. To promote PIC assembly *in vivo*, Mediator cooperates with PIC components, including TFIIB, TFIID, TFIIH and Pol II, through specific protein–protein interactions[5,15,16,18–21]. In line with these different interactions, Mediator coordinates PIC assembly at different steps *in vivo*, and both the head and middle modules have been involved in these functions[17,18]. A further level of regulation of PIC assembly is provided by an interplay between Mediator and the chromatin architecture around promoter regions (mostly including local nucleosome occupancy and positioning)[18,72–77] (FIG. 4). Structural analyses of Mediator–PIC complexes and functional *in vivo* studies complement each other in elucidating the mechanisms of Mediator in PIC assembly.

***Structural insights on Mediator within the PIC.*** As discussed above, the transient and dynamic nature of Mediator interaction with the PIC together with the large size of this multicomponent assembly make the structural analysis of Mediator within the PIC even

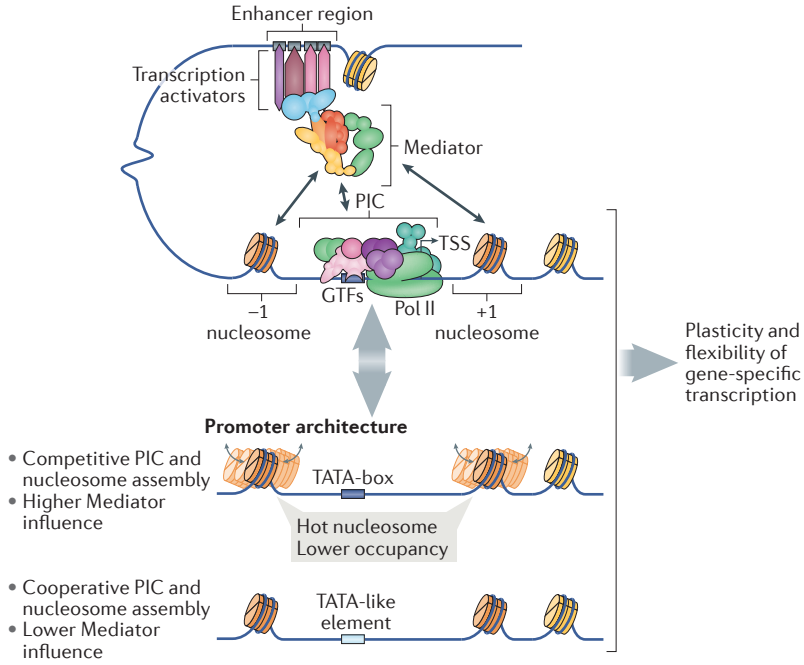


Figure 4 | **Mediator interplay with the preinitiation complex and with promoter architecture.** Mediator of RNA polymerase II transcription (Mediator) stimulates assembly of the preinitiation complex (PIC) and interacts with PIC components, including RNA polymerase II (Pol II), and general transcription factors (GTFs; transcription initiation factor IID (TFIID), TFIIB and TFIIH), as depicted in the top panel of the figure by arrows[5,15–21]. These factors may cooperate in PIC assembly (as exemplified by the interaction between yeast Mediator and TFIID[19–21,70,85]). In addition, cooperative chromatin binding between Mediator and PIC components (specifically TFIID) was also suggested in yeast[61]. As transcription occurs in a chromatin context, interplay between Mediator function and chromatin organization at promoter regions constitutes a relevant aspect of transcription regulation[72–77]. For example, there is a negative correlation between nucleosome occupancy at promoters and Mediator activity, and Mediator has been shown to promote nucleosome eviction. Nucleosome occupancy and dynamics at promoter-flanking regions (+1 and –1 nucleosomes) have been associated with Mediator function in PIC assembly in a manner dependent on promoter sequence composition (that is, the presence of a TATA-box versus a TATA-like element): TATA-box-containing genes with overall lower nucleosome occupancy on transcription start site (TSS)-surrounding regions and with more dynamic –1 and +1 nucleosomes (so-called hot nucleosomes, which are characterized by shorter residence time) more strongly depend on Mediator activity for PIC assembly than the TATA-like-containing promoters, which show less dynamic nucleosomes and higher general nucleosome occupancy[18]. In this regard, competitive PIC and nucleosome assembly was proposed for TATA-box-containing promoters, whereas a cooperative assembly was suggested for promoters with TATA-like elements in yeast[13]. This interplay between Mediator, PIC and promoter architecture could influence flexibility and plasticity of gene-specific transcription. It should be noted that Mediator–chromatin interplay might be complex and might occur at many levels.

more difficult than that of the Mediator complex alone. Mediator physically and functionally interacts with Pol II by multiple contacts, including interactions with several Pol II subunits and the CTD of the largest subunit[5,22,78–82]. Different studies allowed for the visualization of Mediator in complex with Pol II at low resolution and proposed various orientations of these complexes with respect to each other, which may reflect differences in experimental conditions or might be an indication that the formation of the functional complex is a multistep process[27,34,41,83]. Recent studies have addressed the importance of Mediator conformation for its interactions with different partners and the way in which potential conformational changes could influence Mediator function.

The contact of Mediator with Pol II is crucial for its function, and different conclusions were made regarding the conformational changes that Mediator may undergo when it binds to Pol II. For example, no major conformational changes of the yeast PIC were observed upon Mediator binding at the resolution obtained by a recent study[37]. However, a comparison of high-resolution cryo-EM models of fission yeast Mediator alone and in complex with Pol II suggested that conformational changes in Med14 upon Pol II binding, which result in an altered orientation between the Mediator head and middle modules with respect to that seen with free Mediator, are essential for Mediator–Pol II complex formation[38]. A similar Mediator–Pol II orientation was previously reported for budding yeast[30]. However, the observation of this stabilized Mediator–Pol II conformation in cryo-EM experiments does not exclude the possibility that other functional conformations could exist during the assembly of transcription machinery. In humans, the importance of Mediator conformation for its interaction with Pol II was suggested on the basis of EM and biochemical analysis of Mediator point mutants[39]. Mutational analysis of a conserved region within the MED21–MED7 heterodimer, located in the middle module, showed that the affinity of human Mediator for Pol II depends on this region[39]. The relative contributions of multiple contacts between Mediator and Pol II remain to be investigated *in vivo*.

In addition to Mediator–Pol II contacts, recent structural studies have also led to a model for the Mediator core within the PIC in budding yeast and have proposed a model of how Mediator contacts the PIC components[30,37] (Supplementary information S1 (table)). In particular, these studies revealed Mediator–TFIIB contacts, implicating the Mediator head module in the stabilization of these interactions[30,37]. In accordance with the Mediator role in stimulating Pol II CTD phosphorylation by TFIIH, it has also been shown that Mediator–TFIIH contacts are established in proximity to the Pol II CTD[37]. Even though much can be learnt from these structural studies, it is necessary to bear in mind that *in vivo* Mediator is almost certainly highly dynamic and flexible during PIC assembly; similarly, PIC formation is a dynamic, multistep process. Thus, the available structural data on Mediator and PIC conformations may represent only selected conformational states and might only partially correspond to the situation *in vivo*. Accordingly, some differences exist between the structural models of Mediator in the PIC and the *in vivo* data obtained from functional studies[5,18,30,35]. Future studies will certainly resolve these differences. As structural analyses were mainly focused on yeast Mediator, data regarding the conformations of mammalian Mediator in the context of PIC assembly are mostly lacking. Nevertheless, a cryo-EM model for the human Mediator–Pol II–TFIIF complex assembled from purified components provides an important first step towards visualization of human Mediator within the PIC[84].

Enhancer RNAs
Non-coding RNAs that are transcribed from enhancers and contribute to gene looping between enhancers and promoters and thus to gene transcription regulation.

***Interactions and interplay of Mediator with TFIID and TFIIB during PIC assembly.*** Recent studies have revealed the functional interplay between Mediator and PIC components. Several lines of evidence suggest that both human Mediator and yeast Mediator physically interact with TFIID and that these complexes cooperate in PIC assembly *in vitro*[19–21,70,85]. *In vivo*, in yeast, *med17* mutations, which lead to the loss of Mediator activity or specific changes in Mediator function, also result in reduced recruitment of TBP (a TFIID component) to chromatin[17,86,87]. Similarly, depletion of Med14, which links the three main Mediator modules, led to a moderate decrease in occupancy of TFIID subunit 1 (Taf1), another essential TFIID subunit. Conversely, Mediator occupancy was strongly reduced upon Taf1 depletion[61]. Taken together, these observations suggest cooperative chromatin binding between Mediator and TFIID to the yeast genome[61].

On the basis of *in vitro* experiments of yeast Mediator and human Mediator, a similar functional interplay was also proposed for Mediator and TFIIB[88,89]. Furthermore, taking advantage of *in vivo, in vitro* and *in silico* approaches, recent work on *med10*-mutant yeast demonstrated that Mediator and TFIIB interact and that this interaction is important for PIC assembly genome-wide[18]. Mediator–TFIIB contacts were also evident in a structural model of the yeast PIC. Notably, however, the Mediator subunits identified as being involved in this interaction in yeast differ between structural *in vitro* data (which identified Med18 and Med20 within the head module[30]) and functional *in vivo* results (which identified Med10 in the middle module and Med14, which bridges the head, middle and tail modules[18]). This could reflect the dynamics of both Mediator and PIC during PIC assembly but could also be the consequence of only partial PIC assembly in cryo-EM conditions.

***Regulatory influence of chromatin context on Mediator function in PIC assembly.*** PIC assembly occurs in a chromatin context, and transcription initiation is associated with changes in chromatin architecture at promoters. Importantly, nucleosomes undergo extensive remodelling and modification at promoters. These regions are generally depleted of nucleosomes, and a nucleosome-free or nucleosome-depleted region just upstream of the transcription start site (TSS; also known as the +1 position) is generated. This nucleosome-free region is flanked by the +1 and –1 nucleosomes, which are well-positioned with respect to the TSS[90,91]. Thus, it is not surprising that Mediator function in transcription initiation is linked to chromatin architecture at promoters (as shown in both yeast cells and human cells)[72–77]. Notably, it was shown that purified yeast Mediator binds to histones in the nucleosomes[75] and that this Mediator–histone binding depends on histone acetylation[74,77]. There is now evidence from yeast that Mediator may be involved in nucleosome displacement (eviction) to facilitate transcription activation[72,73]. Moreover, a recent analysis of a yeast *med10*-mutant (Med10, as discussed above, is important for interactions with TFIIB)[18] revealed that the role of Mediator in recruiting the main PIC components, including TFIIB, TFIIH and Pol II, was related to the nucleosome occupancy and dynamics around the TSS on a genomic scale[13,92]. It was found that the extent to which Mediator contributes to PIC assembly may be gene specific, in addition to the global impact of Mediator on PIC assembly and transcription. This gene-specific aspect was discovered when comparing genes that had a larger reduction in PIC occupancy on promoters in yeast with mutated Mediator with genes for which this reduction was smaller. This analysis revealed that TATA-box-containing promoters rely more strongly on the function of Mediator to recruit PIC components than promoters with TATA-like elements, and this correlated with lower nucleosome occupancy (including the well-positioned +1 and –1 nucleosomes) and higher nucleosome dynamics at TATA-box-containing promoters than at promoters containing TATA-like elements. This regulatory aspect of Mediator activity could lead to a greater plasticity and flexibility of gene expression[18]. These findings are consistent with a model for PIC assembly that was previously proposed in the wild-type context, suggesting that promoters without the TATA-box rely on the positioning of the +1 nucleosome for the selection of the TSS and PIC assembly[13]. This work also proposed that PIC and nucleosomes are assembled in a competitive manner on promoter regions of TATA-box-containing genes and that their assembly is cooperative for gene promoters with TATA-like elements[13]. It should be noted that another study in yeast previously suggested competition for binding between nucleosomes and PIC components, including Mediator[72]. Taken together, these findings underscore the functional interplay between Mediator, PIC components and the promoter architecture, which is important for transcription regulation, for example, for fine-tuning gene-specific expression (FIG. 4). These results also emphasize the importance of considering the chromatin structure (instead of 'naked' DNA) for analysis of Mediator function and the use of chromatinized templates for *in vitro* studies[93]. In support of this idea, recent work using chromatin assembled *in vivo* as a template for transcription *in vitro* showed more robust transcription of a model yeast gene (*PHO5*) from the chromatin template than from pure DNA, and this increased efficiency was dependent on the presence of nucleosomes, nucleosome remodelling factors (which can change nucleosome positioning and occupancy), histone modifications and Mediator[94]. Several studies in yeast and metazoan cells have also proposed functional interactions of Mediator with chromatin-acting co-activators, including histone acetyltransferases (reviewed in REFS 12,14), providing further evidence that the Mediator–chromatin interplay occurs at many levels during transcription activation.

In addition to transcription initiation, cooperative functions of Mediator and TFIIS in Pol II passage through the +1 nucleosome *in vitro* were evidenced in human cells[76], also showing that the transition of Pol II to active transcription is regulated by Mediator in the chromatin context. Accordingly, Mediator was found to cooperate with TFIIS in PIC assembly, and this component was included in some structural studies of the PIC[37,95,96]. Further investigations are needed to obtain an integrated view of the interplay of Mediator and the PIC with chromatin.

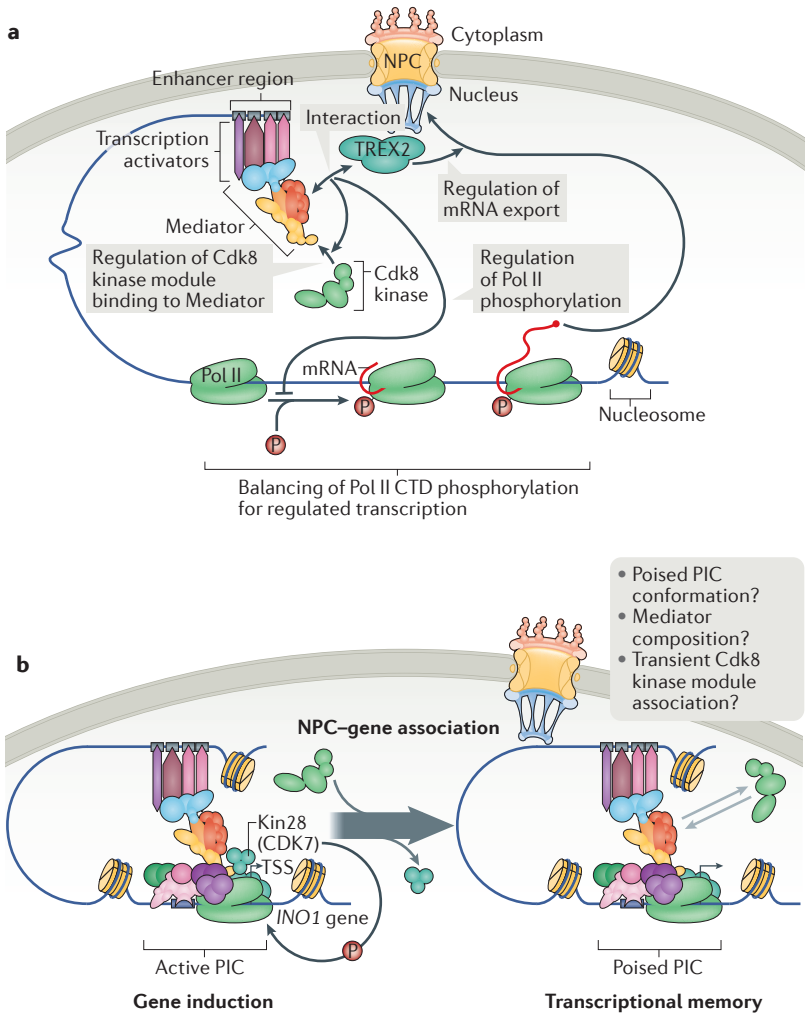


Figure 5 | **Mediator links to the nuclear pore complex. a** | Yeast mediator of RNA polymerase II transcription (Mediator) was recently shown to interact with the transcription-coupled export (TREX2) complex, one of the factors involved in mRNA export from the nucleus, which binds to the nuclear pore complex (NPC)[101]. This interaction between Mediator and the TREX2 complex was proposed to promote the association of the Cdk8 kinase module with Mediator and to decrease the phosphorylation (P) of the carboxy-terminal domain (CTD) of RNA polymerase II (Pol II) at Ser5, thereby balancing the levels of phosphorylated Pol II, which can act in transcription elongation. The precise molecular mechanisms in which Mediator and the TREX2 complex interact and the transcription steps at which these interactions occur remain to be determined. Interestingly, the same protein surface of the TREX2 complex is responsible for its role in transcription regulation though Mediator and for mRNA export. **b** | Mediator has been shown to be involved in transcriptional memory mechanisms. For example, under induction of gene expression in response to stress, an active preinitiation complex (PIC) is assembled, and the regulated gene is activated (left). For some genes, a transcriptional memory phenomenon was described that corresponds to the facilitated reactivation of expression, which is dependent on a previous stimulus and related to the association of the gene with the NPC. It was recently proposed that establishment of transcriptional memory is associated with the presence of a poised PIC on the promoter; this poised PIC is devoid of the cyclin-dependent kinase 7 (CDK7; also known as serine/threonine-protein kinase Kin28 in yeast) subunit of transcription initiation factor IIH (TFIIH) or probably of the entire kinase module of TFIIH (TFIIK), which prevents phosphorylation of Ser5 in the CTD of Pol II (right)[105]. Unlike in the active PIC, Mediator in this poised complex was proposed to contain its Cdk8 kinase module; however, the Cdk8 kinase module association with Mediator in the poised PIC might be only transient (as indicated by the two opposite-facing arrows). The conformation of the poised PIC and the Mediator composition within this assembly remain to be studied. *INO1*, inositol-3-phosphate synthase gene; TSS, transcription start site.

## Connection to the nuclear pore

In addition to its canonical role in transcription activation, Mediator has recently been shown to have a role in genome organization in the nuclear space. Through these mechanisms, Mediator could have further impact on transcription regulation and may be implicated in transcriptional memory (FIG. 5).

***Mediator interacts with the TREX2 complex to couple transcription to mRNA export.*** In eukaryotes, mRNA biogenesis results from fine coordination between pre-mRNA synthesis by Pol II during transcription and a number of co-transcriptional RNA processing events. Phosphorylation of the CTD of Pol II has an essential role in the progression of Pol II through initiation, elongation and termination and in co-transcriptional RNA processing[97]. These processes culminate in a mature mRNA that is exported to the cytoplasm through nuclear pore complexes (NPCs)[98,99]. The conserved transcription-coupled export (TREX2) complex associates with the NPC and is implicated in transcription and mRNA export[100]. A recent study discovered a direct link between Mediator and the TREX2 complex in yeast, which indicates that transcription regulation coordinated by Mediator is connected to NPC-mediated mRNA export[101] (FIG. 5a). By using a combination of genetic, biochemical, structural and functional *in vivo* approaches, this work revealed that the TREX2 complex directly interacts with the middle module of Mediator and that this interaction enables targeting of genes to the NPC. Interaction of the TREX2 complex with Mediator was suggested to be involved in complex regulation of transcription and mRNA transport. The association of the Cdk8 kinase module with the Mediator core is positively regulated by the TREX2 complex. Furthermore, the TREX2 complex was demonstrated to negatively regulate phosphorylation of Ser5 in the CTD of Pol II, thereby balancing the amount of transcriptionally active Pol II. An exact role for Cdk8 in Pol II phosphorylation remains unclear, but this work suggested a joint regulatory effect of Cdk8 and the TREX2 complex on the phosphorylation of Ser5 in the CTD of Pol II[101]. Whether this link between Mediator and the TREX2 complex is conserved in eukaryotes remains to be addressed, and precise molecular mechanisms remain to be uncovered. Further investigations are also needed to understand how these NPC-targeting mechanisms are correlated with Mediator recruitment and changes in its modular organization[62,63]. More generally, this work extends Mediator function from transcription to regulation of nuclear gene positioning. Interestingly, a recent study identified Mediator as one of the key complexes in the yeast genome that is responsible for higher-order chromatin folding, suggesting a broad action of Mediator in regulating global chromatin organization[102].

***Mediator and transcriptional memory.*** A phenomenon known as epigenetic transcriptional memory corresponds to the priming of particular genes for a rapid and strong reactivation under appropriate conditions on the basis of previous cellular stimulus[103,104], and recent evidence suggests that Mediator contributes

to these mechanisms[105] (FIG. 5b). By using the inducible inositol-3-phosphate synthase (*INO1*) gene as a model for transcriptional memory in yeast, the binding of transcription factor Sfl1 was shown to initiate the memory and the targeting of *INO1* to the nuclear periphery. The work suggested that during establishment of memory, which poises *INO1* for re-activation, a Mediator complex composed of all four modules (including the Cdk8 kinase module, in contrast to Cdk8 kinase module-free Mediator, which is recruited to active promoters (see above)) was bound to the promoter region and contributed to recruitment of a poised PIC, which lacks Kin28 and is thus unable to promote phosphorylation of the CTD of Pol II and Pol II promoter escape. This function of Mediator was confirmed for other genes in yeast and shown to be conserved in human cells[105]. Overall, this work suggests that the establishment of a poised PIC versus active PIC assembly is largely regulated by the presence or absence of the Cdk8 kinase module of Mediator. Interestingly, in a system independent of transcription memory, a similar inactive state of the PIC, which included Pol II and Mediator–CDK8 kinase module but which lacked TFIIH, was previously proposed to be assembled in human cells at inactive gene promoters that are regulated by retinoic acid in the absence of retinoic acid ligand[67]. As structural and functional studies in yeast and humans have shown that the dissociation of CDK8 is necessary for Mediator–PIC interactions, PIC assembly and Mediator–Pol II interactions[40,41,106,107] (see above), it remains to be solved how a poised, Pol II-containing PIC could be formed in the presence of the CDK8 kinase module. It is possible that during formation of a poised PIC, Mediator–CDK8 kinase module and PIC components interact differently (employing different conformations) from when an active PIC is established. Of note, there is so far no direct evidence for the simultaneous presence of Pol II and CDK8 kinase module-containing Mediator on chromatin under memory conditions or for their direct interaction within the poised PIC. Thus, it is also possible that the CDK8 kinase module is present in Mediator only transiently when transcriptional memory is being established and later dissociates, enabling recruitment of Pol II and the assembly of a poised PIC. Finally, as transcriptional memory is linked to gene anchoring at NPCs[103,108,109], it remains to be investigated whether interactions between the TREX2 complex and Mediator are involved in this context.

## Mediator and potential therapeutics

Given a key role of Mediator in gene expression, mutations or changes in the expression levels of genes encoding Mediator subunits have been implicated in a number of human diseases[110,111] (BOX 2). Accordingly, Mediator subunits could be potentially used as therapeutic targets, even though at first glance this possibility is difficult to consider owing to the fact that Mediator is generally required for overall cell physiology. Here, two recent advances for potential therapeutic targeting of Mediator are discussed: Mediator kinase inhibition in cancers[112] and disruption of activator–Mediator contacts in fungal infections[113].

***Inhibition of the CDK8 kinase module of Mediator in cancers.*** The CDK8 kinase module of Mediator represents the only characterized enzymatic activity of the Mediator complex. In humans, Mediator kinase module components, in particular, cyclin-dependent kinase 8 (CDK8; which is part of the CDK8 kinase module) and its paralogue CDK19, show strong associations with cancer, and CDK8 is considered an oncogene[110,111,114].

Key determinants of cell identity, including many oncogenes, have been found to be under the control of large clusters of enhancers, called super-enhancers, which show particularly high occupancy of Mediator, other co-regulators and specific transcription factors[58,115]. Consequently, it has been proposed that selective inhibition of oncogenes by disruption of super-enhancers could be a possible strategy for developing cancer therapeutics[57]. As an opposing strategy to target cancer, it has been shown that in cases of acute myeloid leukaemia (AML) specific inhibition of Mediator kinase activity (without any effect on other kinases) boosted the expression of genes controlled by super-enhancers, prominently including tumour-suppressive genes. This work also characterized a selective inhibitor of Mediator kinases, cortistatin A, which showed anti-leukaemic activity with acceptable pharmacokinetics properties in AML mouse models[112]. This provided evidence that Mediator kinases function as negative regulators of super-enhancer-associated transcription and are a promising, druggable target for therapeutic approaches. This study also provided a useful research tool — a selective inhibitor — to study Mediator kinases. Cortistatin A was recently used to identify substrates of Mediator kinases in human cells, opening interesting perspectives for future research[116].

***Disrupting activator–Mediator contacts in fungal infections.*** Except for Mediator kinases, many functions of this complex do not involve enzymatic activity and occur primarily through specific interactions engaging different Mediator subunits. Targeting these specific interaction interfaces, for example, the contacts between activators and Mediator subunits, could be used as a potential therapeutic approach to modulate selected Mediator functions. Mediator interactions with various transcription factors have been structurally characterized[117–119]; this knowledge enabled the identification of a small-molecule inhibitor targeting the interaction between Mediator and a pleiotropic drug resistance transcription factor (Pdr1), the orthologues of which regulate the multidrug resistance pathway in yeast, such as the human pathogen *Candida glabrata*[113]. Inhibition of Mediator–Pdr1 interaction interfered with the expression of Pdr1-regulated genes and restored sensitivity of *C. glabrata* to azole-based anti-fungal drugs. This finding provides a paradigm for studying specific protein–protein interfaces established by Mediator and suggests that disrupting interactions at these interfaces could be used to specifically target transcription events.

## Conclusions and perspectives

With its many subunits, multiple contacts within the nucleus and broad impact on gene expression, Mediator

represents a key target of studies aimed at understanding transcription regulation and transcription-related processes. Studies of Mediator mechanisms and functions have dramatically accelerated in the past few years. However, despite a wealth of mechanistic insights gathered so far, it remains to be fully uncovered how this complex participates in the integration of different regulatory signals *in vivo* in conjunction with other factors. A combination of complementary approaches, including functional *in vivo* studies together with genomic, structural, biochemical, bioinformatics and modelling approaches, will be essential to further expand our understanding of Mediator functions.

Potential mechanisms regulating different aspects of Mediator function have started to emerge that suggest a role of PTMs and the importance of dynamic interactions in the regulation of Mediator activity. A complex regulatory role of the CDK8 kinase module in Mediator function is also evident and should be further analysed, as should the role of Mediator in enhancer–promoter chromatin looping and the regulation of this process[64,65].

Furthermore, Mediator has been shown to function beyond regulating activation of gene expression, and its roles have been extended to postrecruitment steps (after PIC assembly and Pol II recruitment) of transcription[21,67,120,121]. In this context, a recent study that involved single-molecule imaging of transcription sites in live cells proposed a Mediator-dependent mechanism of transcription reinitiation, which produces groups of closely spaced RNA polymerases, called convoys, that together elongate transcripts[122].

The recently discovered link between Mediator and the nuclear pore further extends the function of this complex to RNA metabolism beyond the transcription process per se. Mediator function is likely to be even broader, as suggested by a recent discovery of a link between Mediator and DNA repair[51]. All these functional aspects of Mediator activity should now be studied in more detail and integrated into a more general framework of Mediator regulatory networks.

Overall, studies of Mediator function address fundamental biological questions and allow for a better understanding of normal cell physiology but also reveal important insights into disease-associated dysfunctions and could lead to the development of therapeutic strategies. In this context, studies of mammalian-specific Mediator subunits could provide new insights that could be potentially translated to clinics.


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