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Review

# The Mediator complex and transcription elongation ☆

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ABSTRACT

*Background:* Mediator is an evolutionarily conserved multisubunit RNA polymerase II (Pol II) coregulatory complex. Although Mediator was initially found to play a critical role in the regulation of the initiation of Pol II transcription, recent studies have brought to light an expanded role for Mediator at post-initiation stages of transcription.
*Scope of review:* We provide a brief description of the structure of Mediator and its function in the regulation of Pol II transcription initiation, and we summarize recent findings implicating Mediator in the regulation of various stages of Pol II transcription elongation.
*Major conclusions:* Emerging evidence is revealing new roles for Mediator in nearly all stages of Pol II transcription, including initiation, promoter escape, elongation, pre-mRNA processing, and termination.
*General significance:* Mediator plays a central role in the regulation of gene expression by impacting nearly all stages of mRNA synthesis. This article is part of a Special Issue entitled: RNA polymerase II Transcript Elongation.


## 1. Introduction

Eukaryotic mRNA synthesis is an elaborate process that proceeds with the synthesis and co-transcriptional processing of a pre-mRNA to form a mature, translatable mRNA. Transcription of pre-mRNA is catalyzed by the evolutionarily conserved, multisubunit enzyme RNA polymerase II (Pol II). Transcription takes place through discrete stages referred to as initiation, elongation, and termination, all of which are now recognized as sites for the regulation of gene expression.

Initiation of transcription by Pol II is a complex biochemical reaction governed by the concerted action of a remarkably large collection of general and gene-specific transcription factors. Fundamental to Pol II initiation is the set of five evolutionarily conserved general transcription factors, which are referred to as TFIIB, TFIID, TFIIE, TFIIF, and TFIIH and which comprise the minimum set of transcription factors needed to support synthesis by Pol II of a basal level of accurately initiated transcripts from its promoters *in vitro* [21,23,105]. Mechanistic studies have revealed that Pol II assembles together with the general factors at its promoters to form a stable preinitiation complex that is competent to initiate transcription when provided with ribonucleoside triphosphates [22,23,105].

Efforts to understand how transcription initiation by Pol II and the general factors is regulated by the myriad DNA binding transcription factors known to activate or repress pre-mRNA synthesis led to the discovery of the Mediator, an enormous multisubunit complex that appears to be present exclusively in eukaryotes and to play an integral role in gene regulation [14,15,109]. The Mediator was first identified in *S. cerevisiae* transcription extracts and purified chromatographically by Kornberg and coworkers by its ability to support the activation of Pol II transcription by DNA binding transcription factors, in an enzyme system reconstituted with purified general factors [57,93]. Subsequent investigation of the mechanism of action of Mediator from yeast and higher organisms revealed that Mediator promotes activation of Pol II transcription *via* direct interactions with both DNA binding transcription factors and the Pol II preinitiation complex [5,78,79,93,121]. Further studies have identified an array of Mediator surfaces capable of binding specifically to the transcription activation domains (TADs) of a large number of DNA binding transcription factors [5,13] and to Pol II and several of the general factors [8,9,17,34,48,65]. Through these interactions, Mediator is capable of promoting transcription initiation by Pol II, at least in part by facilitating assembly of functional preinitation complexes [4,18,51,52,130,134].

More recently, experimental evidence implicating Mediator in post-initiation stages of Pol II transcription has emerged. These studies have brought to light roles for Mediator (i) in bypassing or overcoming the activities of factors that negatively regulate elongation [20,50,76], (ii) in recruiting Pol II transcription elongation factors and pre-mRNA processing factors [28,47,90,123] and (iii) in controlling phosphorylation of the heptapeptide repeats in the Pol II C-terminal domain (CTD) [11,28,49,123]. The phosphorylated CTD of elongating Pol II has been shown to function in some steps of chromatin remodeling by acting as a scaffold that recruits histone modifying enzymes to the transcribed region of genes [30,41,68,135]. The phosphorylated

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CTD also plays a central role in coordinating pre-mRNA processing by recruiting many of the enzymes and proteins critical for proper capping, splicing, and polyadenylation of pre-mRNA, as well as for proper nuclear export and localization of mature mRNAs [16,99,138]. Thus, Mediator might participate indirectly in all of these processes by modulating CTD phosphorylation.

In this review, we begin with brief descriptions of the structure of Mediator, its various functional forms, and their roles in the regulation of transcription initiation by Pol II. We devote the remainder of this review to a discussion of recent evidence supporting roles for Mediator in post-initiation stages of Pol II transcription.

## 2. Mediator structure and function

### 2.1. Multiple forms of Mediator

Early attempts to purify Mediator and establish its subunit composition led to the discovery that it can be isolated from cell extracts in multiple, chromatographically distinguishable forms [24,93,121]. Of these forms, the least complex is referred to as the Mediator "core" complex and is composed of more than 20 distinct proteins. Based on biochemical and structural studies, the subunits of the Mediator core complex are organized into at least three modules referred to as the "head," "middle," and "tail." In cells, one fraction of the Mediator core complex is associated with a kinase module, which in *S. cerevisiae* includes the cyclin-dependent kinase CDK8, Cyclin C, and two additional subunits designated MED12 and MED13. Notably, the mammalian kinase module may have evolved a more complex array of functions, since it is composed of Cyclin C and one of two cyclin-dependent kinases CDK8 or CDK19, one of two MED12-like proteins MED12 or MED12L, and one of two MED13-like proteins MED13 or MED13L. Another fraction of the Mediator core complex is free of kinase module, but instead is tightly associated with Pol II in what is sometimes called the Mediator "holoenzyme" complex. The majority of metazoan Mediator core and holoenzyme complexes include an additional subunit, MED26. Finally, a small population of Mediator that contains both kinase module and MED26 can be isolated from mammalian cell extracts [27,109,123].

### 2.2. Mediator function in transcription activation and repression

A major question prompted by the discovery of multiple forms of Mediator was whether the different forms possessed different transcription activities. Initially, it was proposed that Mediator associated with the kinase module might participate exclusively in transcriptional repression. In yeast, results of genetic experiments supported a role for subunits of the kinase module in repression of Pol II transcription [42,45,108,117,129]. In human cells, evidence suggested that Mediator containing the kinase module contributes to repression of Pol II transcription by the DNA binding transcription factor C/EBPβ, whereas Mediator lacking the kinase module but containing MED26 contributes to activation by the same transcription factor [87]. In a related study, the activation domain of the viral transactivator VP16 was reported to activate Pol II transcription in cells through a form of Mediator that is deficient in kinase module [126].

Consistent with these genetic results, core Mediator or MED26-containing Mediator complexes have been shown to support activation of transcription *in vitro* far better than Mediator containing the kinase module [1,62,77,92,118,122]. Indeed, activation of Pol II transcription facilitated by the Mediator core complex can be inhibited by addition of purified kinase module to reactions [59]. Evidence suggests that it does so by binding stably to the Mediator core complex and blocking its interaction with Pol II, either by occluding the Pol II binding site or by an allosteric mechanism [33,59].

Though evidence from these early studies argued that Mediator complexes that do or do not include the kinase module have intrinsically different activities, a litany of new findings has revealed that the story is not so simple. CDK8 kinase activity has recently been shown to be required for activation of transcription of a variety of genes, including a reporter gene activated by the model transcriptional activator GAL4-VP16 [37], genes regulated by the thyroid hormone receptor [6], p53 [29], and Smads [2], and genes in the serum response network [28]. CDK8 has also been shown to participate indirectly in the activation of Pol II transcription by β-catenin, by phosphorylating and inactivating the transcription factor E2F1, which antagonizes β-catenin-dependent gene activation [88]. In addition, the kinase module subunits MED12 and MED13 have been shown to interact with or be required for transcription activation by a variety of DNA binding transcription factors, including β-catenin [56], its *Drosophila* ortholog Pygopus [19], Nanog [125], members of the GATA and RUNX families [40] and yeast Pdr3p [112]. Notably in human cells, direct interactions between the β-catenin activation domain and MED12 have been shown to contribute to Mediator recruitment to genes [56]. Finally, although kinase module and MED26 are often thought to have opposing functions, Boyer and coworkers obtained evidence consistent with the idea that Mediator containing both MED26 and kinase module functions in the repression of transcription of some neuronal genes by recruiting the RE1 silencing transcription factor [27].

## 3. A role for Mediator in Pol II transcription elongation

### 3.1. Mechanisms governing Pol II transcription elongation

Although the initiation stage of Pol II transcription was long regarded as the primary site for the regulation of pre-mRNA synthesis, over the past decade or so it has become clear that the elongation stage of Pol II transcription is also a major site for gene regulation [53,64, for reviews see 67,110,111]. Early evidence that Pol II transcription could be regulated during elongation came with the identification of a handful of genes whose activation was accomplished at least in part by the release of properly initiated, but paused, Pol II into productive elongation. The first examples of genes regulated in this fashion included the *Drosophila heat shock*, human *c-Myc*, and *HIV-1* early genes [7,39,54,63,75,83,95,96,106,119,124,132]. With the development of genomic-scale methods for chromatin immunoprecipitation (ChIP-chip or ChIP-seq), nascent transcript sequencing, and location of transcriptionally engaged Pol II using nuclear run-on assays (GRO-seq), it has become clear that initiated and promoter-proximally paused Pol II can be found 30–50 nucleotides downstream of the start sites of many active and inactive genes, arguing that transcription of a large fraction of genes can be regulated during elongation [25,43,58,91,94,102,103,140].

Although the exact mechanisms underlying the regulated pausing and release of Pol II are not known, features of these processes have been gleaned from a combination of biochemical and genetic experiments, which suggest that Pol II pausing during early elongation is controlled by multiple transcription elongation factors that either negatively or positively influence Pol II. These studies identified DRB-sensitivity inducing factor (DSIF, composed of SPT4 and SPT5) and negative elongation factor (NELF) as two factors that function together to induce Pol II pausing [38,66,86,103,120,127,133,137]. In addition, results of more recent studies identified an additional factor, Gdown1, as a protein that may cooperate with DSIF and NELF to induce Pol II pausing [20]. The role of Gdown1 in Pol II pausing remains controversial, however, as results of another study led to the proposal that it may instead act upstream of initiation, by preventing formation of an initiation-competent preinitiation complex [50].

A variety of evidence argues that the release of Pol II from promoter-proximal pausing requires the cyclin-dependent kinase P-TEFb, which is composed of CDK9 and Cyclin T [72,81,82,98,101,131]. Among the targets of the P-TEFb kinase are the Pol II CTD and the SPT5 subunit of DSIF [82,136]. Although the detailed mechanism by which P-TEFb contributes to the release of Pol II from pausing has not been established,