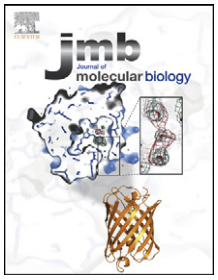

PIC Activation through Functional Interplay between Mediator and TFIIH

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Abstract

The multiprotein Mediator coactivator complex functions in large part by controlling the formation and function of the promoter-bound preinitiation complex (PIC), which consists of RNA polymerase II and general transcription factors. However, precisely how Mediator impacts the PIC, especially post-recruitment, has remained unclear. Here, we have studied Mediator effects on basal transcription in an *in vitro* transcription system reconstituted from purified components. Our results reveal a close functional interplay between Mediator and TFIIH in the early stages of PIC development. We find that under conditions when TFIIH is not normally required for transcription, Mediator actually represses transcription. TFIIH, whose recruitment to the PIC is known to be facilitated by the Mediator, then acts to relieve Mediator-induced repression to generate an active form of the PIC. Gel mobility shift analyses of PICs and characterization of TFIIH preparations carrying mutant XPB translocase subunit further indicate that this relief of repression is achieved through expending energy via ATP hydrolysis, suggesting that it is coupled to TFIIH's established promoter melting activity. Our interpretation of these results is that Mediator functions as an assembly factor that facilitates PIC maturation through its various stages. Whereas the overall effect of the Mediator is to stimulate basal transcription, its initial engagement with the PIC generates a transcriptionally inert PIC intermediate, which necessitates energy expenditure to complete the process.

Introduction

Transcription initiation by RNA polymerase II (Pol II) is dependent on a set of general transcription factors (GTFs) that enable it to site-specifically form a preinitiation complex (PIC) at promoters of mRNA and miRNA genes [1,2]. *In vitro*, functional PICs can be generated by the GTFs TFIIB, TFIID, TFIIE, TFIIF, and TFIIH, which suffice for basal level transcription. These GTFs act in concert to direct and orient Pol II in close proximity to the transcription site and coordinate the melting of double-stranded DNA to allow RNA chain synthesis to begin [3]. Formation and function of the PIC is rate limiting, and it is a major target of transcriptional regulation by gene- and tissue-specific transcription factors [4]. Recent studies have suggested a unified general mechanism for initiation by the three eukaryotic RNA polymerases, which are composed of class-specific, shared, and paralogous subunits [5]. PICs formed by all three polymerases are nucleated by TBP (e.g., as part of TFIID in the case of Pol II), and structural and functional counterparts of all Pol II GTFs except TFIIH have been identified either among the cognate GTFs of Pol I and Pol III or in the non-paralogous subunits of these polymerases. TFIIH is unique among the GTFs in possessing ATP-dependent activities that have been implicated in promoter melting and in possessing a kinase that targets the carboxy terminal domain (CTD) of RPB1, the largest Pol II subunit [6].

Pol II is also unique among the three polymerases in being subject to direct regulation by the multi-subunit Mediator complex [7,8]. The Mediator was initially identified as a coactivator that interfaces between the regulatory transcription factors and the Pol II machinery to activate transcription [7]. Its modular structure, consisting of head, middle, tail, and kinase modules, is ideally suited for such a role.

Mediator can interact with activators, typically via specific tail subunits [9], and with Pol II, via the head and middle [10–12]. However, it is now evident that Mediator does not merely act as a passive conduit for signal transduction between the activators and Pol II. Multiple, new functionalities have now been attributed to the Mediator. These include functions at the level of the chromatin template, in enhancer–promoter communication, and negative roles through the reversibly associating Mediator kinase module [7,8]. Importantly, Mediator can also stimulate basal (activator-independent) transcription [13–16]. Through interactions with a multitude of cofactors and an ability to undergo significant conformational changes, Mediator has the potential to function as an integrative node that delivers precisely calibrated outputs to the transcription machinery [7,8].

Mediator's ability to modulate basal transcription implies a more active role for the complex and suggests that its effects are also exerted after Pol II has been recruited to the promoter, especially since in this context, Mediator entry to the PIC is dependent on the prior anchoring of Pol II to the template [14]. Recent cryo-electron microscopy and cross-linking mass spectrometry (MS) structures of yeast Mediator bound to the PIC have revealed many Mediator–PIC interfaces [11,17]. In addition to the expected contacts with Pol II, the movable jaw of the head module was localized close to the TFIIB B-ribbon domain, in part explaining an earlier observation that excess TFIIB in nuclear extract allows an absolute Mediator requirement for basal transcription in extracts to be bypassed [18,19]. The structures [11,17] also provide insights into how Mediator can facilitate TFIIH recruitment to the PIC. From its proposed location in the PIC, TFIIH would be well positioned to carry out CTD phosphorylation and, presumably, also DNA melting. Functional and physical interactions between TFIIH and Mediator have previously been described. Mediator has been shown to directly recruit TFIIH to certain yeast loci [20] and stimulate its CTD kinase activity [21]. Conversely, TFIIH can be inactivated by the CDK8 subunit of the Mediator kinase module [22]. More recently, it was shown by studies in yeast that TFIIH CTD kinase activity is important, albeit not sufficient, in dissociating Mediator from Pol II as the latter clears the promoters [23,24].

Here, we report that basal transcription in the presence of Mediator entails close coordination between the Mediator and the ATPase-dependent activities of TFIIH. Using a series of *in vitro* assays reconstituted from pure preparations of Pol II, GTFs, and Mediator, we reveal that in the pathway leading to productive transcription, PICs assembled in the presence of the Mediator are initially functionally repressed and that TFIIH's ATPase-dependent activities are critical in the reactivation of the PIC. We show further that in the absence of TFIIH activity, the Mediator-induced block is at the earliest stages of nascent RNA formation. These results provide insights into how Mediator can promote PIC maturation through a series of intermediates with distinct functional properties.

Results

Mediator represses transcription initiation in the absence of TFIIH-dependent ATP hydrolysis

Early studies [25] had revealed an energy requirement for transcription in crude assay systems that was subsequently traced to the ATPase-dependent DNA melting activity in the XPB subunit of TFIIH (also known as p89 and ERCC3 and as Ssl2 in yeast) [26]. It was further shown that this activity of TFIIH is dispensable when transcription was carried out from supercoiled templates in the presence of Pol II and the other GTFs [27,28]. We now asked how the inclusion of the Mediator into PICs reconstituted from highly pure factor preparations might impact energy-dependent steps in early transcription.

Whereas our previous studies have focused on the role of Mediator in activated transcription [29,30], here we have focused on establishing a simplified assay system to monitor Mediator effects on basal transcription. For this purpose, we used a G_{15}-STOP template (Fig. 1a), whose core promoter elements were derived from the adenovirus major late (ML) core promoter and in which tandem G-residues were inserted 15 nt downstream of the transcription site [30]. In the presence of the nucleotides CpA, UTP, and CTP and the chain blocker 3′ *O*-methyl GTP, the G_{15}-STOP template yields both a 16-mer "productive" transcript that is made by Pol II that has escaped (Fig. 1a) and also a set of abortive and slippage-induced products [31]. This template thus allows a more direct readout of events occurring at very early stages of basal transcription. Furthermore, the 16-mer stretch is A-free such that energy dependence of the reaction can be readily controlled by providing or withholding ATP (or deoxyATP, which is interchangeably used in some experiments).

Linearized G_{15}-STOP template was first transcribed in *in vitro* transcription reactions reconstituted with purified and carefully titrated preparations of human Pol II, TBP, TFIIB, TFIIE, TFIIF, and TFIIH [32]. As expected, under these conditions, productive transcription of the 16-mer was completely dependent on TFIIH (Fig. 1b, lane 3 *versus* lane 1). A circa 4-mer abortive product was, however, observed in the absence of added TFIIH (Fig. 1b, lane 1). While we cannot rule out that trace amounts of TFIIH, which are below MS and Western blotting detection limits, are present in our Pol II preparation, especially given that TFIIH is required only in