CDK8: A Transcriptional CDK

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Abbreviations: ALP, agonist-induced linker phosphorylation; BMP, bone morphogenetic protein; CAK, CDK-activating kinase; CDK, cyclin-dependent kinase; ChIP, chromatin immunoprecipitation; CTD, carboxy-terminal domain of RNAPII; GTF, general transcription factor; IEG, immediate early gene; LEF, lymphoid enhancer-binding factor; MAPK, mitogen-activated protein kinase; PIC, pre-initiation complex; P-TEFb, positive transcription elongation factor b; RNAPII, RNA polymerase II; R-SMAD, receptor-regulated SMAD; SMAD, SMA/mothers against decapentaplegic; STAGA, SPT3-TAF II31-GCN5L acetylase; T3, thyroid hormone; TAF, TBP associated factor; TBP, TATA binding protein; tCDK, transcriptional CDK; TCF, T-cell factor; TFTC, TBP-free TAF, TAF-containing complex; TGFβ, transforming growth factor-beta; TR, thyroid hormone receptor

CDK8 belongs to a group of cyclin-dependent kinases involved in transcriptional regulation from yeast to mammals. CDK8 associates with the Mediator complex, but functions outside of Mediator are also likely. Historically, CDK8 has been described mostly as a transcriptional repressor, but a growing body of research provides unequivocal evidence for various roles of CDK8 in gene activation. Several transcriptional programs of biomedical importance employ CDK8 as a coactivator, including the p53 network, the Wnt/β-catenin pathway, the serum response network, and those governed by SMADs and the thyroid hormone receptor, thus highlighting the importance of further investigation into this enigmatic transcriptional regulator.

CDK8 is a nuclear serine-threonine kinase that functions as a transcriptional regulator. Most of what is known about CDK8 results from its facultative association with the Mediator complex, but since only a fraction of CDK8 is associated with Mediator in cells, roles outside of this complex are also possible.1,3 Several phosphorylation targets for CDK8 have been identified, including the RNA polymerase II (RNAPII) C-terminal domain (CTD), histone H3, subunits of general transcription factors (GTFs) and certain transactivators.4-15 but how these phosphorylation events contribute to the overall biological activity of CDK8 remains ill defined. Research in yeast and metazoan cells has depicted CDK8 as a co-repressor.1,2,4,6,7,9,10,14,17 However, a recent flurry of independent reports demonstrates that CDK8 also plays positive roles in transcription.2,8,11,18-23 In the past two years CDK8 has been described as a coactivator in molecular pathways of biomedical relevance, including the β-catenin pathway.22,23

Phylogenetic analysis of the human kinome places CDK8 among the cyclin-dependent family of protein kinases.26,27 Although CDKs were originally characterized by their role in regulation of the cell cycle, several members of this family have direct functions in regulation of RNAPII activity. The best known of these ‘transcriptional CDKs’ (tCDKs) are CDK7, CDK8 and CDK9. Common features among these tCDKs are: (1) They are members of multiprotein transcription regulatory complexes. CDK8 is part of the CDK-module of Mediator.28 CDK7 is a subunit of TFIIH, a GTF component of the Pre-Initiation Complex (PIC), but it also retains some function in cell cycle regulation by acting as the CDK-activating kinase (CAK).30 CDK9 is the catalytic subunit of positive transcription elongation factor b (P-TEFb), a widespread regulator of RNAPII elongation.31-33 (2) The tCDKs can phosphorylate the RNAPII CTD. This domain is composed of heptad repeats (consensus sequence YSPTSPS) that undergo several phosphorylation events thought to regulate association with RNAPII-interacting factors throughout the transcription cycle.34 CDK9 can phosphorylate Ser2 and Ser5 within the CTD repeats in vitro, but its in vivo contributions remain ill defined. CDK7 has been demonstrated to phosphorylate both Ser5 and Ser7 in vitro, but recent chemical genetics experiments indicate that its major contribution in vivo is Ser7 phosphorylation.4-6,28,35-38 CDK9 is the major Ser2 kinase, but it can also contribute to Ser5 phosphorylation both in vitro and in vivo.3,39-41 (3) tCDKs are subject to
CDK8: A Subunit of Mediator

Mediator is a large complex composed of 25–30 proteins arranged in structural modules that is thought to act as a molecular bridge between DNA-binding transcription factors and RNAPII (reviewed in refs. 43 and 44). Mediator can restore activator-dependent transcription in cell free assays and is considered a quasi-universal regulator of RNAPII transcription. Although numerous interactions of Mediator with DNA-binding proteins and GTFs have been identified, the precise mechanism by which Mediator transduces regulatory signals to RNAPII remains poorly characterized. CDK8 associates in a dynamic fashion with the rest of Mediator as part of a four-subunit subcomplex dubbed the CDK-module (CDK8, cyclin C, MED12 and MED13).1,49–51 Mediator complexes lacking or containing the CDK-module will be referred to here as core Mediator and CDK8-Mediator, respectively (containing the CDK-module will be referred to here as core Mediator.1,2)

Figure 1. Hierarchical map of CDK8 interactions. CDK8 interacts directly with subunits of the CDK-module, which connects to core Mediator via MED13. Analysis of endogenous CDK-module purified away from core Mediator revealed interactions with GCN1L, TriC, MED14 (likely the interaction surface in core Mediator for MED13) and P-TEFb. In turn, CDK8-Mediator (but not core Mediator) interacts with TRRAP and GCN5L to form T/G Mediator, which can catalyze phospho-acetylation of histone H3. Core Mediator displays largely mutually exclusive interactions with the CDK-module and RNAPII. Mediator interacts with multiple GTFs. Activators can target subunits of core Mediator or the CDK-module.

reconstituted human CDK-module by the Taatjes lab has generated several insights into CDK8 regulation.1,2 First, it was found that MED12—but not MED13—was required for kinase activity of the reconstituted CDK8-module toward the RNAPII CTD and other targets.2 Second, it was revealed that MED13 mediates the interaction between the CDK-module and the rest of Mediator, likely via MED14.1,2 and that MED13 itself can be phosphorylated by CDK8.2 Third, association of the CDK-module with core Mediator enabled CDK8 to phosphorylate histone H3 on chromatin.1 These observations are significant as they suggest that not only is CDK8 regulated at the level of kinase activity by association with other subunits of the module, but also by substrate accessibility via association with core Mediator. Mass spectrometry analysis of purified endogenous CDK-module revealed its association with additional factors including GCN1L and the TriC chaperonin (Fig. 1), the latter of which may be able to sequester the free CDK-module.2 Most intriguingly, unlike the recombinant CDK-module, the endogenous CDK-module could not phosphorylate RNAPII CTD, suggesting that these novel interactors might serve a regulatory role, perhaps by inhibiting the activity of the free CDK-module until its association with core Mediator.

These studies reveal several possible layers of CDK8 regulation and prompt key questions. What are the roles of GCN1L and TriC in overall CDK8 biological activity? Is there a differential impact of MED12 versus MED12L or MED13 versus MED13L on the regulation of CDK8? What is the role of MED13 phosphorylation and does it affect association of the CDK-module with core Mediator? Is CDK19 regulated in a similar fashion to CDK8? Future studies combining biochemical reconstitution and genetic dissection in cells will be required to address these issues.
CDK8: First Hyde, then Jekyll

Historically, reports documenting negative roles for CDK8 in transcriptional control have outnumbered those depicting it as coactivator. Notable evidence for CDK8 acting as a co-repressor can be summarized as follows: (1) SRB10 (the CDK8 homolog in yeast) was shown to repress transcription in vitro when allowed to phosphorylate the RNAPII CTD prior to PIC assembly.1 (2) Human CDK8 was demonstrated to repress transcription via phosphorylation and inactivation of the cyclin H subunit of TFIH.7 (3) SRB10 was shown to inactivate yeast transactivators by triggering their nuclear export or degradation.9,12 (4) Microarray analysis in yeast showed that whereas core Mediator subunits played mostly positive roles in global gene expression, subunits of the CDK-module behaved largely as negative regulators.17 (5) Human CDK8-Mediator was found to be less active than core Mediator in co-activation assays in vitro56,57 and addition of recombinant CDK-module blocked the coactivator function of core Mediator.1 This repressive action was independent of CDK8 kinase activity and was due to disruption of Mediator-RNAPII interactions likely by MED12-MED13.3 Mutually exclusive interactions between Mediator and RNAPII or the CDK-module were also observed in structural studies of S. pombe Mediator;14 and in extensive MudPIT analysis of human Mediator variants.58,59 (6) A correlation between gene activation and an exchange of CDK8-Mediator for core Mediator was observed during: (a) MAPK-dependent activation of the transcription factor C/EBPβ,15 and (b) Retinoic acid-induced activation of the RARβ2 gene.16

While it is clear that CDK8 can negatively affect transcription in some contexts, the following sections describe the evidence for positive roles of CDK8 in transcription. We present several prominent studies in detail to highlight the distinct mechanisms by which CDK8 can positively affect gene activation. These include kinase-dependent promotion of re-initiation events and scaffold formation, coactivator recruitment, and transcription-coupled activator turnover, elongation factor recruitment and chromatin modification.

SRB10 Acts as a Positive Regulator of Transcription in Yeast by Multiple Mechanisms

While most of the early SRB10 literature established its repressive functions, a minority of reports prevented a uniform view on this factor. First, a simple correlation between SRB10 binding to promoters and gene silencing was refuted by genome-wide chromatin immunoprecipitation (ChIP) studies showing that SRB10 is present on both active and inactive genes in vivo.58,59 Second, while SRB10 inactivates some transcription factors, it can also enhance the activity of others. For example, it was shown that SRB10-dependent phosphorylation of GAL4 is required for proper activation of galactose-inducible genes via a mechanism involving transactivation-coupled turnover.11,60 Third, in contrast to the early studies showing SRB10 repression via RNAPII CTD phosphorylation, later work showed that SRB10 collaborates with KIN28 (the yeast CDK7 homolog) to promote RNAPII re-initiation.8 Following PIC formation and an initial round of transcription, it is thought that subsequent rounds of RNAPII binding and promoter clearance are enabled via a ‘scaffold complex’ that is composed of a subset of Mediator subunits and GTFs that remain bound at the promoter.61 In elegant chemical genetic experiments using analog-sensitive isoforms of SRB10 and KIN28, the Hahn lab established a cooperative positive role for both kinases in scaffold formation and re-initiation in vitro, and intragenic RNAPII transit at active genes in vivo.8 Formation of the scaffold complex is an ATP-dependent process.61 Interestingly, it was shown that SRB10 phosphorylates two subunits of TFIID (BDF1 and TAF2) and KIN28 phosphorylates two subunits of Mediator (MED4 and RGR1/MED14).8 However, the role of these phosphorylation events has not yet been defined. In sum, these studies establish a positive role for SRB10 in yeast. Does CDK8 employ similar mechanisms in metazoans?

CDK8 is a Positive Regulator of p53-Dependent Transcription

Early studies in mammalian cells indicated recruitment of CDK8 along with core Mediator subunits to genes regulated by the aryl hydrocarbon receptor and serum response factors, but the role of CDK8 in transcriptional activation at these loci was not explored at the time. Our research into the mechanism of regulation of the p53 target gene p21 (CDKN1A) led to a similar observation of CDK8 recruitment upon activation, which prompted us to directly test the impact of CDK8 on gene activity.21,25 In response to p53 activation by UV-mediated DNA damage, p21 undergoes limited rounds of transcription followed by rapid inactivation and loss of pre-loaded RNAPII.64 In contrast, p53-activating agents such as Nutlin-3 lead to sustained p21 activation over many hours without loss of promoter-bound RNAPII.21,25,64,65 We found that this unequal regulation of p21 is not determined by differential p53 binding or p53-mediated histone acetylation, but correlates instead with the assembly of stimulus-specific transcriptional complexes at the promoter.21,25,64 Whereas certain core Mediator subunits are recruited to p21 regardless of the p53-activating agent, CDK8, Cyclin C and MED12 are recruited exclusively during conditions of sustained activation.21,25 Similarly, while binding of TBP and TFIIA occurs in all scenarios, association of TFIIB and TFIIIF increases only under sustained promoter activity.21,25,64 Such differential behavior among Mediator subunits and GTFs could be indicative of remnant scaffold complexes after transient activation versus ‘complete’ PIC assemblies maintained during conditions of sustained re-initiation (Fig. 2).

Importantly, we have also observed association of CDK8 with several other p53 target loci during activation, and microarray experiments using cell lines stably expressing shRNAs against CDK8 showed that CDK8 is mostly a positive regulator of transcription within this network (Donner and Espinosa, unpublished results). Do these observations apply only to p53 target genes or do they illuminate a more widespread positive role for CDK8 in gene activation?
CDK8 is Required for Thyroid Hormone Receptor-Dependent Transcription

Mediator is a key coactivator for the thyroid hormone receptors (TRs), the nuclear hormone receptors that mediate the physiological actions of the thyroid hormone (T3). In a recent report, Belakavadi and Fondell demonstrate that CDK8 cooperates with core Mediator to promote TR-dependent gene activation. They found that TR recruits subunits of both core Mediator and the CDK-module along with RNAPII to immobilized promoter templates in a T3-dependent manner. After addition of ATP, they observed that, whereas RNAPII, CDK8, TFIIF and TFIIB quickly disassociated from the template, subunits of core Mediator and TFIID remained bound (Fig. 2), forming a structure reminiscent of both the yeast scaffold complex and the transcriptional complex observed at the p21 promoter after transient activation by UV. In contrast, the TR-recruited complex formed prior to ATP addition mimics the complete assembly observed on p21 during sustained activation (Fig. 2).

Furthermore, using a number of cell-based assays, Belakavadi and Fondell demonstrated that CDK8 and its kinase activity are important for TR-dependent transcription in vivo. First, ChIP assays showed recruitment of CDK8, MED12 and MED13 along with core Mediator subunits and RNAPII to the TR-target gene DIOI. Second, knockdown of CDK8 or cyclin C impaired T3-dependent activation of DIOI to the same extent as knockdown of core Mediator subunits. Finally, they showed that the deleterious effects of CDK8 depletion could be rescued with a wild type CDK8, but not a kinase-dead mutant.

Overall, the findings of the Hahn, Espinosa and Fondell labs suggest a conserved role for SSB10/CDK8 in stimulation of the transcription machinery at the level of scaffold complex formation and transcription re-initiation (Fig. 2). However, as discussed below, CDK8 has also been shown to function as a...
Serine to alanine substitution of the ALP sites within SMAD5 and SMAD3 attenuates their ability to activate transcription, suggesting ALP may be necessary for interaction with coactivators. Indeed, ALP of SMAD1 was demonstrated to be required for interaction with the coactivator protein YAP. Second, ALP primes activated R-SMADs for turnover by acting as a binding site for specific E3 ubiquitin ligases such as Smurf1. Accordingly, knockdown of either CDK8 or CDK9 led to slower degradation of activated R-SMADs following removal of agonist. Thus, CDK8 and CDK9 couple the transactivation potential of the R-SMADs with their turnover to ensure precise temporal control of TGFβ- and BMP-responsive genes. This role for CDK8 parallels that observed for SRB10 in the galactose-induced transcriptional program, where phosphorylation of Gal4 by SRB10 triggered its degradation, which in turn was required for full gene activation. Transactivation-coupled turnover has become a recurrent theme in transcriptional regulation, and CDK8 may play a widespread role in this phenomena. Along these lines, the Jones lab reported that CDK8 is recruited by the coactivator Mastermind to Notch-responsive genes, where it targeted the Notch intracellular domain for turnover.

CDK8 Enhances Smad Transactivation by Enhancing Co-Activator Recruitment

Activation of the membrane receptors for TGFβ and BMP leads to C-terminal phosphorylation of the receptor-regulated SMAD family of transcription factors (R-SMADs) and subsequent translocation to the nucleus where they control the expression of hundreds of genes. Conversely, antagonistic signaling through stress- and cell cycle-regulated kinases (MAPks and CDK2/4, respectively) results in phosphorylation of the R-SMADs in their linker region leading to cytoplasmic retention and degradation. A recent report describes an additional layer of regulation of R-SMAD activity. Whereas antagonist-driven linker phosphorylation occurs in the cytoplasm, the Massague group discovered that TGFβ and BMP activation lead to linker phosphorylation in the nucleus by CDK8 and CDK9 (this event was dubbed agonist-induced linker phosphorylation or ALP) (Fig. 3). The functional consequence of ALP is twofold. First, ALP is necessary for R-SMADs to fully activate their target genes. Serine to alanine substitution of the ALP sites within SMAD5 and SMAD3 attenuates their ability to activate transcription, suggesting ALP may be necessary for interaction with coactivators. Indeed, ALP of SMAD1 was demonstrated to be required for interaction with the coactivator protein YAP. Second, ALP primes activated R-SMADs for turnover by acting as a binding site for specific E3 ubiquitin ligases such as Smurf1. Accordingly, knockdown of either CDK8 or CDK9 led to slower degradation of activated R-SMADs following removal of agonist. Thus, CDK8 and CDK9 couple the transactivation potential of the R-SMADs with their turnover to ensure precise temporal control of TGFβ- and BMP-responsive genes. This role for CDK8 parallels that observed for SRB10 in the galactose-induced transcriptional program, where phosphorylation of Gal4 by SRB10 triggered its degradation, which in turn was required for full gene activation. Transactivation-coupled turnover has become a recurrent theme in transcriptional regulation, and CDK8 may play a widespread role in this phenomena. Along these lines, the Jones lab reported that CDK8 is recruited by the coactivator Mastermind to Notch-responsive genes, where it targeted the Notch intracellular domain for turnover.
CDK8 is an Oncogene Required for β-Catenin-Dependent Transcription

Recent studies show that CDK8 functions as a potent oncogene in colon cancer cells through effects on Wnt/β-catenin signaling. Upon activation of the canonical Wnt pathway, β-catenin is stabilized and translocated into the nucleus where it forms a ternary complex with TCF/LEF transcription factors to activate genes involved in cell proliferation such as MYC and Cyclin D1. Mutations in the Wnt pathway leading to abnormally high levels of β-catenin activity are thought to drive several malignancies, most prominently colorectal cancer. CDK8 was identified during two shRNA loss-of-function screens as one of only nine genes that were required for both colon cancer cell proliferation and β-catenin-driven transcription. Significantly, of these nine genes, CDK8 was the only one exhibiting frequent copy number gain in colon cancers. Suppression of CDK8 expression inhibited proliferation of colon cancer cell lines exhibiting increased levels of CDK8, while overexpression of wild type, but not kinase-dead, CDK8 induced transformation of NIH 3T3 cells. CDK8 was found to associate with the MYC promoter and knockdown of MED12 or Cyclin C had similar effects to CDK8 knockdown, suggesting that CDK8 is working as part of the CDK-module to enhance the β-catenin transcriptional program. What is the mechanism by which CDK8 promotes β-catenin-dependent transcription?

Earlier work from the Boyer lab established that the β-catenin transactivation domain interacts with MED12 and is able to recruit Mediator to Wnt-responsive genes in vivo. MED12 knockdown inhibited β-catenin transactivation potential and MED12 isoforms carrying mutations in the β-catenin-binding domain blocked expression of Wnt-target promoters. Overall, these results provide a mechanism for recruitment of CDK8 via the CDK-module to enhance β-catenin transactivation. It remains to be determined if CDK8 can directly target components of the β-catenin/TCF/LEF complex or if it functions instead via effects on the general transcriptional apparatus.

Interestingly, an independent study by the Dyson lab has further emphasized the importance of CDK8 for β-catenin activity in a process involving the transcriptional regulator E2F1. In human cells, E2F1 strongly repressed β-catenin-dependent transcription and induced degradation of β-catenin. CDK8 was found to suppress these effects, and was able to bind and phosphorylate E2F1 in vitro, suggesting that CDK8 inhibits the repressive activity of E2F1 towards β-catenin. Furthermore, ChIP analysis demonstrated that both CDK8 and E2F1 were present at the MYC promoter where they would be able to exert their opposing effects on β-catenin. Thus it appears that CDK8 acts directly and indirectly to both enhance β-catenin transcriptional activity and to release it from the inhibitory effect of E2F1.

CDK8-Mediator Promotes RNAPII Elongation within the Serum Response Network

Interestingly, whereas CDK8 was required for β-catenin-dependent transformation, Firestein et al. reported that overexpression of a dominant negative version of TCF had only a partial effect on CDK8-induced transformation, suggesting that CDK8 plays additional oncogenic roles outside the Wnt pathway. Given the prominent role of Ras/MAPK-dependent signaling in tumorigenesis, we postulated that CDK8 could have functions within the MAPK-regulated transcriptional program. Using isogenic cell lines with normal or reduced levels of CDK8, we found that CDK8 is indeed a potent positive regulator of immediate early genes (IEGs), those which are strongly and transiently induced within minutes of growth factor stimulation. ChIP analysis showed that CDK8 is recruited to these genes during activation as part of CDK8-Mediator (Fig. 3). Remarkably, we found that CDK8 depletion did not affect recruitment of RNAPII to IEGs or overall RNAPII intragenic occupancy, but instead caused a clear decrease in RNAPII CTD phosphorylation at both Ser2 and Ser5 at the genes examined without affecting total cellular levels of either mark. Nuclear run-on experiments demonstrated that CDK8 depletion leads to the appearance of slower elongation complexes. Further ChIP analysis showed that CDK8 knockdown impaired recruitment of CDK7 and CDK9. While a role for Mediator in recruitment of TFIIH had been previously reported in yeast, a role for Mediator in P-TEFb recruitment was unprecedented. Serendipitously, MudPIT analysis of Mediator versus CDK8-Mediator complexes by the Taatjes lab showed that P-TEFb interacts preferentially with the free CDK-module. In sum, these results indicate that Mediator, via CDK8, regulates post-RNAPII recruitment steps within the serum response network. The notion that Mediator regulates elongations steps was first introduced by the Berk lab. In their pioneering work, they identified MED23 as the interface by which the serum-activated factor ELK1 recruits Mediator, including CDK8, to serum responsive genes. Although abolishing Mediator recruitment drastically impaired transcriptional activation, a significant fraction of RNAPII remained bound to the promoter leading them to conclude that Mediator affected both recruitment and post-recruitment steps. Although these efforts did not test the role of CDK8 itself, it nevertheless established the concept that Mediator could promote late steps of the transcription cycle.

CDK8 Catalyzes Histone Modifications Associated with Transcriptional Activation

Biochemical isolation and MudPIT analysis of human Mediator complexes in the Taatjes lab led to the discovery that the GCN5L acetyl-transferase and its associated polypeptide TRRAP bind stably to CDK8-Mediator, but not core Mediator. This novel complex, dubbed T/G Mediator (Fig. 1), was not active in co-activation assays, but did catalyze tandem phosphoacetylation of histone H3 at Ser10/Lys14, a mark associated with gene activation. Furthermore, ChIP assays showed that CDK8-Mediator and TRRAP are present at active p53- and serum-responsive genes. Additionally, CDK8 knockdown led to lower cellular levels of the tandem modification. TRRAP and GCN5L are sub-units of the STAGA/TFTC histone acetyl-transferase complex
in humans. Interestingly, the yeast CDK-module (SRB8-11) functions with the biochemically similar SAGA complex to co-activate Gal4-activated promoters.46 The mechanism by which tandem S10P/K14Ac promotes gene activation is not fully understood, but it has been proposed that this mark leads to recruitment of P-TEFb via BRD4, which binds both acetylated lysines and P-TEFb (reviewed in ref. 77). Intriguingly, BRD4 also interacts with Mediator regardless of the presence of the CDK-module.20 Thus, in theory, T/G Mediator could facilitate P-TEFb recruitment by at least three mechanisms: physical interaction with the CDK-module and/or BRD4, and, indirectly, via K14 acetylation. It is possible that these alternative mechanisms are used in a gene-specific manner. Indeed, our studies on serum response genes indicate that CDK8-mediated P-TEFb recruitment at these loci occurs independently of effects on histone acetylation.20

**A Working Model for CDK8 in Transcriptional Activation**

Although there is not yet a clear picture of why CDK8 acts positively in some cases and negatively in others, it seems likely that the net influence of CDK8 will be governed by substrate accessibility and by gene- and stimulus-specific susceptibility to regulation at each stage of the transcription cycle.

Overall, we can identify several biochemical steps during the transcription cycle at which CDK8 could promote gene activity. A generic model integrating all these steps is shown in Figure 4; however, it remains to be determined which of these events occur simultaneously at a particular locus and to what extent this model is qualified by differences between yeast and mammalian factors. According to this model, activators recruit Mediator via interaction with subunits of core Mediator (as in the case of p53 and TR) or the CDK-module (as observed for β-catenin). Recruitment of Mediator completes PIC assembly, and, in the case of promoters not carrying pre-loaded RNAPII, induces RNAPII recruitment. Next, in an ATP-dependent process, CDK8 and CDK7 collaborate to trigger the dissociation of the complete PIC into a scaffold complex and an early elongation complex. This may involve phosphorylation of TFIID, Mediator subunits and/or RNAPII CTD. In any case, RNAPII leaves the promoter as do the CDK-module and TFIIF. Although TFIIB also dissociates from the promoter, it is unclear if it accompanies RNAPII into intragenic regions. RNAPII release from the scaffold may be assisted by disruption of Mediator-RNAPII interactions by MED12-3. Detachment of the CDK-module from core Mediator may be facilitated by phosphorylation of MEDI2-3. Detachment of the CDK-module from core Mediator may be facilitated by phosphorylation of MEDI3 and MEDI4, the subunits connecting the CDK-module to core Mediator, by CDK8 and CDK7, respectively. TFIIB, TFIID, TFIIH and to a lesser extent TFIIE remain promoter-bound with core Mediator subunits to facilitate subsequent rounds of re-initiation. Finally, P-TEFb is recruited to facilitate transcriptional elongation, either via physical interactions with the CDK-module or BRD4. Alternatively, BRD4/P-TEFb recruitment may be reinforced by CDK8/GCN5L-mediated H3 phospho-acetylation. We hope this working model will inspire future research aimed at testing its veracity.

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