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REVIEW

Regulatory functions of the Mediator kinases CDK8 and CDK19

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ABSTRACT

The Mediator-associated kinases CDK8 and CDK19 function in the context of three additional proteins: CCNC and MED12, which activate CDK8/CDK19 kinase function, and MED13, which enables their association with the Mediator complex. The Mediator kinases affect RNA polymerase II (pol II) transcription indirectly, through phosphorylation of transcription factors and by controlling Mediator structure and function. In this review, we discuss cellular roles of the Mediator kinases and mechanisms that enable their biological functions. We focus on sequence-specific, DNA-binding transcription factors and other Mediator kinase substrates, and how CDK8 or CDK19 may enable metabolic and transcriptional reprogramming through enhancers and chromatin looping. We also summarize Mediator kinase inhibitors and their therapeutic potential. Throughout, we note conserved and divergent functions between yeast and mammalian CDK8, and highlight many aspects of kinase module function that remain enigmatic, ranging from potential roles in pol II promoter-proximal pausing to liquid-liquid phase separation.

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Introduction

The CDK8 kinase exists in a 600 kDa complex known as the CDK8 module, which consists of four proteins (CDK8, CCNC, MED12, MED13). The CDK8 module associates with regulatory sites on a genome-wide scale [1–3], and global targeting of the CDK8 module appears to reflect its association with the 26-subunit Mediator complex[4]. CDK8 module–Mediator association is reversible [5–7] but stable, and distinct populations of "CDK8-Mediator" complexes can be biochemically purified [8,9]. The Mediator–CDK8 module interaction occurs via MED13 [10,11] and an undefined set of Mediator subunits. A paralog of CDK8, called CDK19, emerged in vertebrates and has high sequence similarity to CDK8, including near-identical cyclin binding and kinase domains. Comparatively little is known about CDK19; however, it appears to assemble into an analogous "CDK19 module" (i.e. CDK19, CCNC, MED12, MED13)[12]. In addition to CDK19, paralogs for MED12 and MED13 (MED12L and MED13L) emerged in vertebrates. Unlike CDK19, the MED12 and MED13 paralogs are more divergent in sequence, with only 59% and 53% sequence identity, respectively. The kinase module paralogs MED12L and MED13L associate in a mutually exclusive fashion with MED12 and MED13 [12], and their potential functional distinctions remain unclear.

CDK8 is considered both an oncogene [13–15] and a tumor suppressor[16], indicative of its cell-type and context-specific roles. Through mechanisms that remain incompletely understood, human CDK8 promotes cell growth via the serum response pathway [17] and also functions to maintain both tumors and embryonic stem cells in an undifferentiated state[18]. Further highlighting the basic role for CDK8 in cell proliferation and development, knockout of CDK8 in flies or mice is embryonic lethal [19,20].

In this review, we describe the functional roles of the Mediator kinases CDK8 and CDK19 and propose speculative models for how they might regulate pol II transcription in various contexts. We start with transcriptional reprogramming and enhancer-promoter looping and then transition to metabolism. Next, we discuss small molecule inhibitors, which have yielded valuable insights about CDK8 and CDK19 function. We conclude with sections devoted to the mechanism of action of Mediator kinases, in the context of the four-subunit kinase module and the 29-subunit CDK8-Mediator complex.

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Mediator kinases, enhancers, and transcriptional reprogramming

Sequence-specific, DNA-binding transcription factors (TFs) are major drivers of cell physiology and cell state[21]. As examples of their biological influence, fibroblasts can be converted into myotubes upon expression of a single TF, MyoD[22], and various combinations of TFs can reprogram somatic cells to a pluripotent state [23–27]. Consistent with these themes, specific sets of TFs define each cell type and enforce expression of cell type-specific genes [28–32]. Current models of how TFs establish and maintain cell type-specific gene expression patterns involve TF binding to clustered sites at enhancer and promoter regions. This concentrated, localized TF binding recruits factors such as Mediator and cohesin to help form and stabilize enhancer-promoter loops [2,3]; these loops, in turn, promote high-level expression of cell type-specific genes [33,34], many of which include the lineage-specific TFs required to initiate the feed-forward cascade[35]. The importance of enhancer-promoter interactions (via formation of stable chromatin loops) is underscored by the fact that looped architectures change during developmental transitions [36–38] and their disruption is pathogenic [39,40]. ChIP-Seq data suggest Mediator occupies promoter and enhancer regions genome-wide, and is especially abundant at super-enhancers [41,42]. In fact, occupancy of the Mediator subunit MED1 is considered a marker for super-enhancers, along with H3K27Ac and BRD4 [43]. Super-enhancers represent clusters of enhancers that form interconnected hubs with other enhancer and promoter sequences to support high levels of gene expression[44].

The Mediator kinases CDK8 and CDK19 regulate TF function through phosphorylation [45,46]; in addition, Mediator appears to be required for expression of most, if not all, pol II transcripts in mammalian genomes, and ChIP-Seq experiments indicate that the CDK8 module co-localizes with Mediator genome-wide [2,3], including at super-enhancers [41,47,48]. Based upon these observations, a reasonable expectation is that disruption of CDK8 and/or CDK19 function would markedly impact global gene expression patterns. Contrary to these expectations, knockdown of CDK8 or CDK19 protein levels [1,17] or inhibition of CDK8 and CDK19 kinase activity [46,48,49] does not globally affect steady-state mRNA levels; rather, only subsets of genes are affected that vary with context (e.g. hypoxia) or cell type.

How can these results be reconciled? Although the mechanistic roles of Mediator kinases remain enigmatic (see below), we speculate that CDK8 and CDK19 regulate pol II transcription, in part, through enhancers and enhancer-promoter communication (Figure 1). Whereas the human genome contains an estimated fifty- to sixty-thousand enhancers[50], only a subset of these will be "active" in any given cell type[51], and this is dependent upon chromatin structure and TF binding[52]. Enhancers are cell type-specific and active enhancers reflect the lineage-specific TFs that bind enhancer sequences [38,53]. Although the process of enhancer activation remains incompletely understood, it coincides with TF binding and bidirectional transcription of enhancer RNAs (eRNAs) [54]. Accordingly, bidirectional eRNA transcription is cell type-specific[55]. Interestingly, changes in eRNA transcription appear to be a seminal event in response to signaling cascades [56–58]. This rapid, bidirectional eRNA transcriptional response, which is triggered by stimulus-specific TFs[55], can occur within minutes of a stimulus, and correlates with a re-organization of enhancer-promoter contacts and a "reprogramming" of gene expression networks [59,60].

The CDK8 module may be an essential component of this rapid enhancer response, based upon a number of observations [61]. CDK8 occupies enhancer elements genome-wide[48], and CDK8 (and/or CDK19) phosphorylates TFs that bind enhancer elements[62]. TF phosphorylation by Mediator kinases has been shown to alter TF activity [45,46]; TF activity, in turn, correlates with expression of bidirectional, enhancer-associated eRNAs [54,55,57]. The expression of eRNAs also correlates with formation of enhancer-promoter loops[63], and may function, at least in part, through direct interactions with the CDK8-Mediator complex[64]. Each of these activities (formation of enhancer-promoter loops, eRNA transcription, TF phosphorylation) can contribute to the establishment of new gene expression programs, whether during cellular differentiation or in response to signaling cascades.

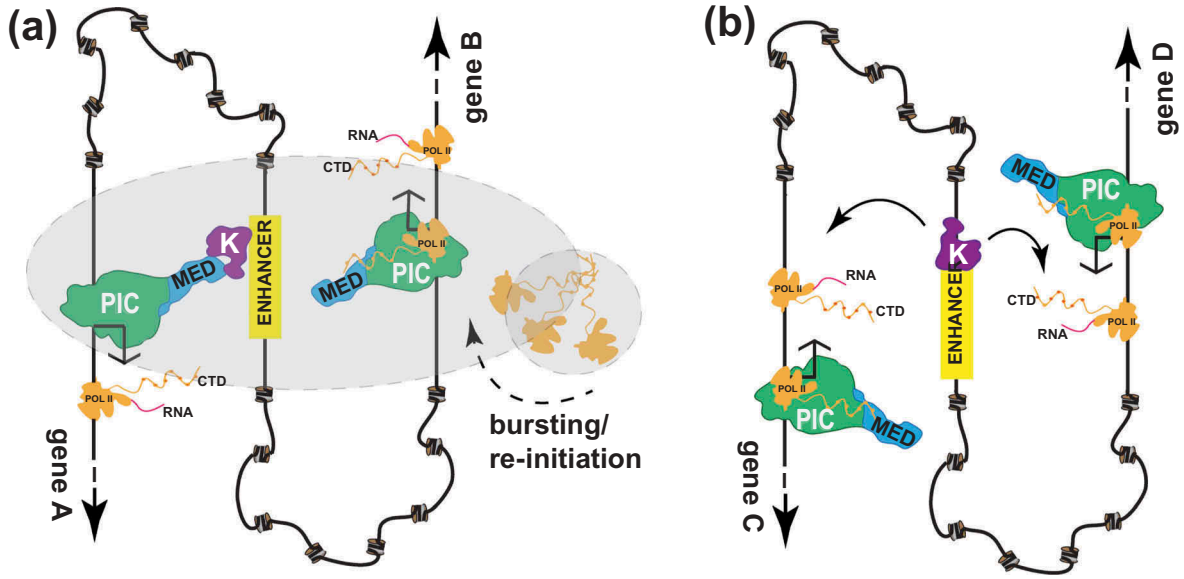


Figure 1. Speculative models for CDK8 or CDK19 module function at mammalian enhancers. **a)** CDK8 or CDK19 module ("K") association with an enhancer (e.g. via TF binding) enables its interaction with promoters that are juxtaposed via chromatin loops. This co-localization may be facilitated by liquid-liquid phase separation (LLPS)[137], which is represented by grey shading. At gene A (left), CDK8/19 module–Mediator binding occurs after pol II clears the promoter. Whereas this interaction prevents rapid re-initiation by another pol II complex, promoter-bound CDK8-Mediator can regulate pol II pausing and/or elongation, through physical or functional interactions with the Super-Elongation Complex (SEC) or other factors [17,62]. At gene B (right), transcriptional bursting is depicted[137], in which multiple pol II complexes initiate in succession[70]. This process appears to be Mediator-dependent[148]. The CDK8/19 module does not associate with Mediator at this promoter, as CDK8 module–Mediator binding is mutually exclusive with Mediator–pol II binding [146,147]. Note that at a different point in time, the situation could be switched, with CDK8/19 module association with gene B and gene A undergoing transcriptional bursting. **b)** An alternative model enables the CDK8/19 module to function independently of Mediator[117]; the enhancer-bound kinase module may regulate transcription elongation via its juxtaposition with elongating pol II complexes at co-localized genes. In support of this model, enhancers have been observed to track with elongating pol II[149], and the CDK8 module appears to positively regulate transcription elongation[17].

Given the potential role of enhancer transcription (e.g. eRNAs) in directing gene expression programs [56,65,66], combined with known roles for Mediator in enhancer-promoter communication [2,59,64], we hypothesize that CDK8 and CDK19 may regulate expression of eRNAs as part of Mediator's overall regulatory regime.

Copy number estimates of CDK8 module subunits (except for CDK8 itself) are generally 5–10 times lower than Mediator subunits, as determined by quantitative proteomics in HeLa cells[67]. Paralogs CDK19, MED12L and MED13L are even less abundant. This suggests (but does not prove) a more specialized role for the CDK8 module. The copy numbers for MED12, MED13, and CCNC (ca. 3000 to 5000) are roughly consistent with the number of active cell type-specific enhancers and with the estimated number of pol II foci in HeLa cells[68]. Emerging evidence suggests that a single enhancer is capable of simultaneous activation of multiple genes (Figure 1) [69,70]; thus, reduced copy numbers of CDK8 module subunits (vs. Mediator, which is about 10x less abundant than pol II) [67] is consistent with this model.

The model proposed in Figure 1 is speculative and further work is needed to test and develop these concepts. An implication of the model is a role for CDK8 and/or CDK19 in transcriptional reprogramming. That is, activation of gene networks that were previously dormant. By this definition, transcriptional reprogramming occurs during developmental and cell state transitions or in response to extracellular stimuli (e.g. cytokines or hormones). In support of this model, Mediator kinase inhibition or CDK8/CDK19 knockdown typically has minimal impact on basal gene expression and generally is well-tolerated in cells under normal growth conditions [1,17,48,71]. By contrast, activation of gene sets in response to stress [1,72–74] or developmental cues [17,20] shows a dependence upon CDK8 or CDK19. This effect likely derives from their kinase function, which may support the establishment of new transcriptional programs through TF phosphorylation.

To a degree, data from yeast support the findings in mammalian cells; however, the mechanistic connections are limited by the distinct regulatory requirements between yeast and mammals. Presumably as a consequence of their smaller and more compact genomes, yeast lack enhancer elements that define mammalian gene regulation [75] and that enable regulatory interactions at extended distances in mammalian genomes [65,76,77]. Accordingly, yeast generally lack bidirectional eRNA transcription [78,79] and chromatin architectural proteins such as CTCF. Instead, yeast possess upstream activating sequences (UAS) that are only a few hundred base pairs upstream of the promoter. Despite these differences, genetic experiments have implicated yeast (*S. cerevisiae*) Mediator and its CDK8 ortholog in UAS-dependent regulation of transcription[80]. Furthermore, data suggest that UAS-bound kinase module transiently interacts with Mediator at promoters [81–83].

CDK8 and transcriptional memory

Related to transcriptional reprogramming is the concept of transcriptional memory, in which a transcriptional response to a stimulus is more rapid in cells that have previously been exposed to the stimulus. A study by the Brickner group, in *S. cerevisiae*, showed that loss of the CDK8 ortholog Srb10/Ssn3 negatively affected transcriptional memory at the *INO1* locus [84]. In particular, wild-type cells showed more rapid transcriptional responses upon re-introduction of the stimulus. This "memory" persisted for 3–4 cell generations (about 6 hours). Importantly, Brickner and co-workers demonstrated similar results in human (HeLa) cells upon stimulation with IFNγ, which led the authors to conclude that CDK8 may be a conserved regulator of transcriptional memory [84].

Whereas the mechanisms remain to be established, it is plausible that the CDK8–Mediator complex may help establish long-distance enhancer-promoter loops in human cells [2,3], with potentially a simpler bridged interaction between the *INO1* promoter and its UAS in *S. cerevisiae*. Formation of enhancer-promoter loops has been observed prior to gene activation by extracellular stimuli [52] and prior to expression of lineage-specific genes during mammalian development [85]. Transcriptional memory may also require chromatin modifications[86]. Reactivation of the *INO1* gene in *S. cerevisiae* correlated with dimethylation of histone H3K4; in an unrelated study, Srb10/Ssn3 kinase activity was genetically linked to Set1-dependent H3K4 methylation in *S. cerevisiae* [87]. In human cells, CDK8 is able to phosphorylate histone H3S10, perhaps concurrently with acetylation of H3K14 [9]. Establishment of such chromatin marks may represent a mechanism by which transcriptional memory or transcriptional reprogramming could be enforced.

Mediator kinases and metabolism

Metabolites represent the biochemicals that – together with proteins and nucleic acids – comprise the entire repertoire of molecules in a cell. As such, metabolic changes are arguably as important as gene expression changes or proteome changes in controlling cell fate or disease pathogenesis [88,89], and metabolic changes are widely recognized as drivers of cancer and cell differentiation [90–94]. Cancer cells rely heavily upon glycolysis (commonly known as the Warburg effect) [88,90] whereas differentiated, non-proliferating cells divert metabolites toward oxidative phosphorylation[91].

The Mediator kinase CDK8 has diverse and conserved links to metabolism. CDK8 orthologs in *Drosophila* and yeast have been linked to lipid and glucose metabolism and regulation of cellular responses to nutrient depletion [95–102]. In the yeast *S. cerevisiae*, the Young lab completed gene expression analyses (microarray; normal vs. starved state) with a kinase-inactive mutant CDK8 ortholog, Srb10[103]. This revealed that about 3% of genes were regulated by Srb10 kinase activity, and that normal kinase function repressed their expression. Most genes affected by kinase-dead Srb10 were involved in cellular response to starvation or nutrient stress[103]. Other studies in *S. cerevisiae* have established Srb10 kinase-dependent regulation of DNA-binding TFs that regulate metabolic pathways [104–108], suggesting ancient origins for Mediator kinases in response to nutrient stress.

In mammalian cells, knockdown experiments have shown a role for the CDK8 protein in the induction of serum response genes[17], and chemical genetics has revealed that CDK8 kinase activity up-regulates

expression of glycolytic enzymes in HCT116 cells [109]. This up-regulation of glycolysis is consistent with a role for CDK8 as an oncogene [13,14] and for stem cell maintenance[18]. In addition, CDK8 is important for cellular response to hypoxia [1] which, like serum response, induces extensive metabolic changes. Moreover, CDK8 protein levels correlate with mTOR signaling in mammals[110], and recent data from our lab [62,72] and others link Mediator kinase function to cholesterol metabolism[99].

The ability of Mediator kinases to regulate cell metabolism likely derives from phosphorylation of DNA-binding TFs, such as SREBP [99] and STAT1 [45,48]. However, other Mediator kinase substrates that do not affect transcription may contribute, such as direct modification of metabolic enzymes and/or signaling proteins (e.g. IPMK or SIRT1)[62].

In mammals, the kinase module subunit MED13 has profound effects on metabolism *in vivo*[111]. Using mouse models, the Olson lab has demonstrated that systemic metabolic processes are sensitive to Med13 protein levels in the heart[112]. For instance, cardiac-specific Med13 over-expression improved insulin sensitivity and conferred resistance to obesity, whereas cardiac-specific Med13 deletion had the opposite effect. Remarkably, mice with cardiac-specific Med13 over-expression showed no changes in food uptake or physical activity, but exhibited increased oxygen consumption and carbon dioxide production[112]. This suggests increased flux through the citric acid cycle and enhanced electron transport, processes that take place in mitochondria. Subsequent studies revealed these metabolic effects manifested in liver and adipose tissue; that is, increased lipid uptake, β-oxidation, and mitochondrial content was observed in these tissues when Med13 was over-expressed in the heart[113]. These results help explain the lean phenotype of these mice[112]. Parabiosis experiments implicated a secreted, cardiac-derived circulating factor but its identity remains unknown [113,114]. Mechanistically, Med13 appears to exert these effects by affecting gene expression patterns [111]. Because MED13 represents the key interface between the kinase module and Mediator [10,11], its effects on metabolism *in vivo* may depend in part upon targeting Mediator kinases to specific genomic loci via its interaction with the Mediator complex.

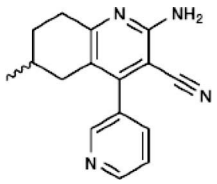
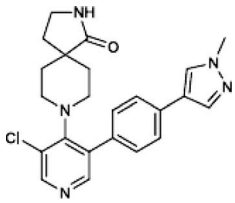
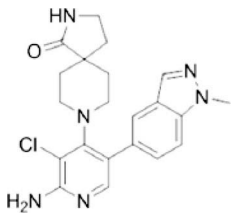
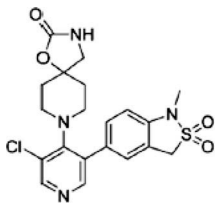
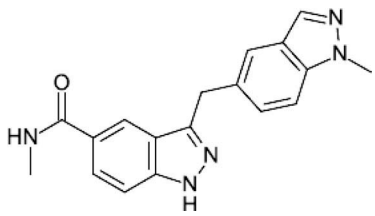
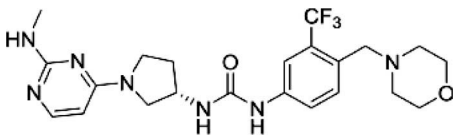
Mediator kinase inhibitors and their therapeutic potential

A number of Mediator kinase inhibitors have been discovered and developed over the past few years, and these are summarized in Table 1. Among them, the natural product cortistatin A (CA) stands apart based upon its potency and selectivity for CDK8 and CDK19[48]. Initial isolation of CA identified other kinases, such as ROCK1, as potential targets in addition to CDK8 and CDK19[115]. A limitation of the initial screen was that it measured binding to the kinase protein itself[116]; that is, CDK8 was not tested as an active kinase[115]. CDK8 lacks measurable kinase activity unless it associates with CCNC, and CCNC + MED12 increases CDK8 activity about 30-fold[117].

In vitro kinase experiments with CA and the four-subunit, 600 kDa CDK8 module (MED12, MED13, CDK8, CCNC) revealed an IC_{50} of about 10nM, whereas the Kd of CA for the CDK8–CCNC dimer was determined to be 0.2nM (Table 1)[48]. Crystal structure data revealed the structural basis for CA selectivity, further verified by kinome profiling assays (KiNativ and ProQinase; collectively testing about 400 distinct kinases). This selectivity for CDK8 and CDK19 was observed even when CA was evaluated at 1μM, which is 5000-times the measured Kd. Importantly, CDK8 and CDK19 were verified as the biologically relevant targets in MOLM-14 cells via site-directed mutagenesis (W105M) that enabled CDK8 or CDK19 resistance to CA inhibition[48]. Although these data cannot rule out CA binding to non-kinase targets, its potency and selectivity for CDK8 and CDK19 is unmatched compared with other chemical probes currently available (Table 1).

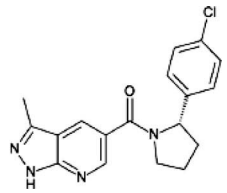
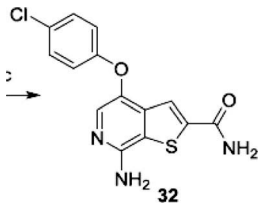
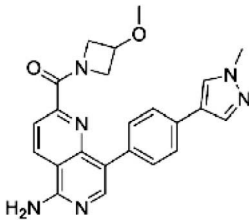
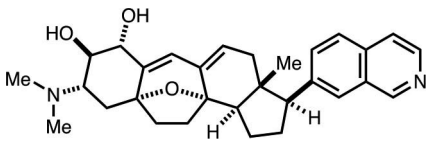
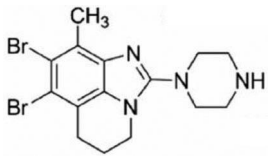
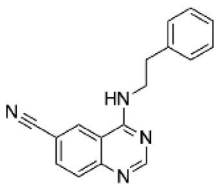
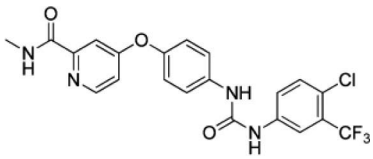
Another chemical probe that stands out is JH-XI-10–02, with a structural scaffold based upon the CA steroid core[118]. Whereas this core structure selectively targets JH-XI-10–02 to CDK8 and CDK19, JH-XI-10–02 also contains pomalidomide tethered via a flexible polyethylene glycol linker. As an analog of thalidomide, pomalidomide enables recruitment of Cereblon, an E3 ubiquitin ligase[119]. This, in turn, triggers ubiquitination of the targeted protein (i.e. CDK8 or CDK19) and subsequent degradation by the proteasome. This

Table 1. Mediator kinase inhibitors as chemical probes Data listed are for CDK8, but similar results are reported for CDK19 unless otherwise stated. All compounds are reversible inhibitors (i.e. non-covalent) and bind competitively with ATP. Each compound has varying levels of off-target effects and the extent of off-target kinase inhibition (i.e. kinases other than CDK8 or CDK19) was tested more rigorously with some compounds compared with others.

Name	structure	IC_{50}[a]	other data	REF
BRD6989		500nM *in vitro kinase w/CDK8-CCNC*	selective for CDK8 vs. CDK19	49
CCT251545		5nM *in vitro kinase w/ CDK8-CCNC*	K_d = 3.8nM (CDK8-CCNC) IC_{50} = 65nM (luciferase reporter) PDB: 5BNJ	122
CCT251921		2.3nM *Lanthascreen or reporter displacement (CDK8-CCNC)*	IC_{50} ~ 20 nM (luciferase reporter) PDB: 5HBJ	153
compound 2[b]		1.8nM *Lanthascreen or reporter displacement (CDK8-CCNC)*	GI_{50} = 2nM+ (many lines tested) IC_{50} ~ 10nM (luciferase reporter)	123
compound 18		10nM *Reporter displacement* 53nM *Lanthascreen (CDK8-CCNC)*	IC_{50} = 65nM (luciferase reporter)	154
compound 20		17.4nM *Lanthascreen (CDK8-CCNC)*	IC_{50} = 6.5nM (luciferase reporter) PDB: 5HVY	155

(*Continued*)

Table 1. (Continued).

Name	structure	IC_{50}[a]	other data	REF
compound 25		2.6nM *Lanthascreen or reporter displacement (CDK8-CCNC)*	IC_{50} = 6.5nM (luciferase reporter) PDB: 5IDN	156
compound 32		1.5nM *assay method unclear*	GI_{50} = 5.4μM* (HCT116) selective for CDK8 vs. CDK19	71
compound 51		5.1nM *Lanthascreen or reporter displacement (CDK8-CCNC)*	IC_{50} = 7.2nM (luciferase reporter)	157
cortistatin A		12nM *in vitro kinase w/ CDK8 module*[#] 100μM ATP	K_d = 0.2nM (CDK8-CCNC) GI_{50} = 5nM (MOLM-14) PDB: 4CRL	48
SEL120-34A		4.4nM *in vitro kinase w/ CDK8-CCNC* 10μM ATP	K_d = 3nM (CDK8-CCNC) GI_{50} ~ 12nM (SKNO-1)	158
senexin A		280nM *in vitro kinase w/ CDK8-CCNC*	K_d ~ 800nM (CDK8-CCNC)	159
sorafenib		32.5nM *Lanthascreen (CDK8-CCNC)*	K_d = 100nM (CDK8-CCNC) PDB: 3RGF	155 160

[a]The IC_{50} results determined from kinase assays will be dependent on ATP concentration, with higher [ATP] yielding higher IC_{50} values. [ATP] used in the assays is noted if reported.

[b]Other compounds with similar activity were tested in this study.

*In this study, the GI_{50} was determined to be due to an off-target effect based upon studies in CDK8 and/or CDK19 knockout cell lines.

[#]CDK8 module = CDK8, CCNC, MED12, MED13

PROteolysis TArgeting Chimera (PROTAC) strategy of selectively targeting enzymes for proteolytic degradation has emerged as a promising therapeutic approach, in part because a single bi-functional molecule can repeatedly target its substrate for degradation [120]. Furthermore, with respect to the Mediator kinases, markedly different effects have been observed upon kinase inhibition vs. knockdown of CDK8 or CDK19 protein levels [62]. Thus, PROTACs should yield therapeutic advantages that are distinct from targeted Mediator kinase inhibition. PROTACs may be especially relevant for therapeutic targeting of CDK19, which has shown kinase-independent effects in the regulation of p53 response[72].

A growing number of studies directly link CDK8 and CDK8 module subunits to specific types of cancer (reviewed in [121]) and the development of selective inhibitors of CDK8 and CDK19 has helped establish Mediator kinases as therapeutic targets [48,49,150]. Studies that have used a variety of methods to disrupt CDK8 and/or CDK19 function have generally shown that kinase inhibition does not markedly affect normal cellular function [46,48,73]. Instead, Mediator kinase activity appears more critical for transcriptional "reprogramming" in response to developmental or environmental cues (see above). These characteristics may be beneficial in the clinic, as they suggest that Mediator kinase inhibitors may be well-tolerated *in vivo*. Available pre-clinical data in mouse models support this conclusion in some cases[48], but other challenges such as bioavailability and therapeutic index remain to be resolved[151].

Inhibition of Mediator kinases may also have therapeutic value in preventing multi-drug resistance, a near-universal obstacle in cancer medicine. The development of drug resistance requires reprogramming of signaling cascades and gene expression networks to circumvent the vulnerability exploited by the treatment[122]. The CDK8 module subunit MED12 has been implicated in multi-drug resistance in numerous cancer cell types [123,124]. In colon, lung, and other cancer cell types, its role appears to involve TGFβ signaling; specifically, under conditions of drug selection, MED12 knockdown induced TGFβR2 expression and triggered activation of MEK/ERK pathways[123]. This was observed in response to a variety of chemotherapeutics, ranging from cisplatin and 5-fluorouracil to more targeted ALK (crizotinib) and BRAF (vemurafenib) inhibitors [123]. Notably, MED12 was still required for proliferation in these resistant cell lines and near-complete depletion was broadly cytotoxic.

Because MED12 activates human CDK8 and CDK19 kinase function [117,125], the development of multi-drug resistance may be due, in part, to changes in CDK8 and/or CDK19 kinase activity. Inhibitors of other transcriptional CDKs, such as CDK7 and CDK12, have been shown to prevent the onset of multi-drug resistance[126], presumably because inhibition of CDK7 and/or CDK12 hinders the establishment of new gene expression programs. Likewise, Mediator kinase inhibitors may prevent the development of multi-drug resistance by similar means; however, this hypothesis remains to be tested.

CDK8 and CDK19 enzymatic activity vs. scaffold function

Among proteins with enzymatic activity, it is commonly observed that the cellular or physiological effects of enzyme inhibition do not match protein depletion or deletion. For transcriptional regulators, this disparity has been demonstrated across different types of enzymes, such as kinases [103,127], acetyltransferases[128], and ubiquitin ligases[129]. These results underscore the importance of the physical presence of an enzyme for structural or scaffolding purposes. In agreement, several studies have documented markedly distinct effects upon knockdown or knockout of CDK8 or CDK19 vs. targeted kinase inhibition [62,72]. Because enzymes are typically components of multi-subunit assemblies, removal by knockdown or knockout can adversely affect the stability or function of the other subunits. This has been consistently observed for the CDK8 module[130]. For example, knockdown of CDK8 can reduce MED12 protein levels in HCT116 cells [17,61]. In contrast to what has been observed in mammalian cells, genetic disruption of srb10/cdk8 kinase activity in yeast phenocopies srb10/cdk8 deletion mutants [103]. Thus, structural/scaffolding roles for

Mediator kinases do not appear to be conserved in yeast.

Kinase module roles in transcription remain enigmatic

As kinases, CDK8 and CDK19 impact cellular function primarily (but not entirely) through protein phosphorylation. The large number of proteins whose phosphorylation state is affected by CDK8 and/or CDK19 implies a complex and elaborate regulatory network [62]. This complexity is compounded by the fact that Mediator kinase substrates are likely to change as a function of cell type or physiological context, and the functional consequences of Mediator kinase-dependent phosphorylation are hard to predict.

The cell type-specific and context-dependent functions of Mediator kinases offer intriguing parallels with DNA-binding TFs, which are also expressed in cell type- and context-specific ways [29]. Sequence-specific, DNA-binding TFs represent a major class of proteins that are targeted by CDK8 and CDK19 [62], and the cell-type and context-specific functions for Mediator kinases could reflect CDK8/19-dependent TF phosphorylation. In a few well-studied cases, CDK8 and/or CDK19-dependent TF phosphorylation has been shown to alter TF activity [45,46,99]. Future experiments will no doubt provide additional insights, but it is notable that even two-fold changes in the level of lineage-specifying TFs can alter cell state and induce differentiation [131,132]. Similarly, two-fold changes in TF activity could have the same effect; thus, it is plausible that TF modification by Mediator kinases underlies many of its biological functions.

Beyond DNA-binding TFs, CDK8 and/or CDK19 phosphorylate other general transcription factors, including TFIID (TAF10), the Super Elongation Complex (SEC; AFF4), NELF (NELFA), pol II (POLR2M), and Mediator itself. Chromatin remodelers and modifiers (e.g. SETD1A, CHD4, KDM3A) were also identified as high-confidence Mediator kinase substrates in HCT116 cells [62]. The functional consequences of these phosphorylation events (if any) remain to be characterized; however, several studies have implicated CDK8 in the regulation of pol II pausing and elongation [1,17,48], suggesting that phosphorylation of proteins such as AFF4, NELFA, or POLR2M may control their function. Yeast lack NELF and the pol II subunit POLR2M, and *S. cerevisiae* pol II does not appear to be regulated by promoter-proximal pausing [133,134]. Thus, mechanistic insights from yeast are expected to be limited in this case.

The kinase activity of CDK8 and CDK19 is regulated by CCNC and MED12 [117,125,135], and ablation of CCNC in mouse cells prevents CDK8 association with MED12 and MED13 [130]. These results suggest a complex network of interactions within the CDK8 module; such interactions are likely similar with CDK19, but perhaps not identical. Although CDK19 is nearly indistinguishable in its kinase domain, about 120 residues in its C-terminus have diverged from CDK8. Low-resolution structural data [10,11,136] and *in vitro* mechanistic assays have shown that CDK8 module association with Mediator blocks pol II association to inhibit transcription initiation or re-initiation events, invoking a "checkpoint" model for transcription[10]. Both MED12 and MED13 appear important for this checkpoint activity. Whereas a role for MED12 remains unclear, structural and biochemical studies have established that MED13 physically links the CDK8 module to Mediator [10]. This role for MED13 is conserved going back to yeast [11].

As a "molecular switch" that governs Mediator–pol II interaction, the association of the CDK8 module with Mediator may have profound functional consequences. How CDK8 module–Mediator association and dissociation is regulated is a key question that remains incompletely resolved. MED13 contains phospho-degron motifs that trigger its ubiquitination and degradation, and this can impact relative levels of CDK8 module–Mediator association in human cells [5]. However, other means to control CDK8 module association and dissociation with Mediator on more rapid time scales (e.g. seconds to minutes) are likely to exist, but our mechanistic understanding is limited [6,7]. Reversible post-translational modifications could play a role, as well as potential tethering via enhancer-bound TFs or eRNA binding [64]. A tethering role could promote re-association with Mediator by maintaining a high local concentration of the CDK8 module near sites of active

transcription. Association of the CDK8 module in phase separated condensates is conceptually similar, and is discussed further below.

Mediator kinase module and liquid-liquid phase separation

An additional means by which the CDK8 module may regulate pol II transcription is via liquid-liquid phase separation (LLPS) [137]. LLPS represents a phenomenon in which proteins and/or nucleic acids achieve higher local concentrations and form "membrane-less organelles" with properties distinct from bulk solvent[138]. Sequence characteristics of proteins that form phase-separated droplets include intrinsically disordered regions, of which Mediator [139] and the CDK8 module have in abundance (Figure 2). Although the molecular forces that contribute to LLPS in biological contexts remain incompletely understood, some basic principles are beginning to be established[140].

The potential for Mediator [141,142] and the CDK8 module to phase separate may facilitate functionally relevant interactions or may help target the CDK8 module (or CDK8-Mediator) to key regulatory loci. Human CDK8 module subunits – in particular MED12, MED13, and their paralogs MED12L and MED13L – possess domains that are predicted to be intrinsically disordered (Figure 2), suggesting the CDK8 module or CDK8-Mediator may have evolved to phase separate. Human MED12 and MED13 are substantially larger than their yeast counterparts, with intrinsically disordered regions that are not conserved.

Although the relative contribution of LLPS to transcription regulation remains to be determined, it is notable that the pol II C-terminal domain (CTD) is a low-complexity, intrinsically disordered sequence that readily forms phase separated droplets *in vitro* [143] and in cells [144,145]. In the yeast *S. cerevisiae*, the pol II CTD consists of 26 heptapeptide repeats of the sequence YSPTSPS. In humans, the pol II CTD is twice the length (52 repeats), and its additional 26 heptapeptide repeats contain nine positively charged residues (mostly lysine) that are spaced throughout the distal 26 repeats. Phase separation is driven in part by pi-cation interactions[140], and the positive charges present in the distal half of the human CTD may promote such interactions with tyrosines spaced throughout the CTD sequence. In agreement, human pol II shows greater propensity to undergo LLPS *in vitro* and in cells, compared with *S. cerevisiae* pol II[144].

It remains to be established whether Mediator, CDK8-Mediator, or the CDK8 module can form phase separated droplets *in vitro*; however, a large

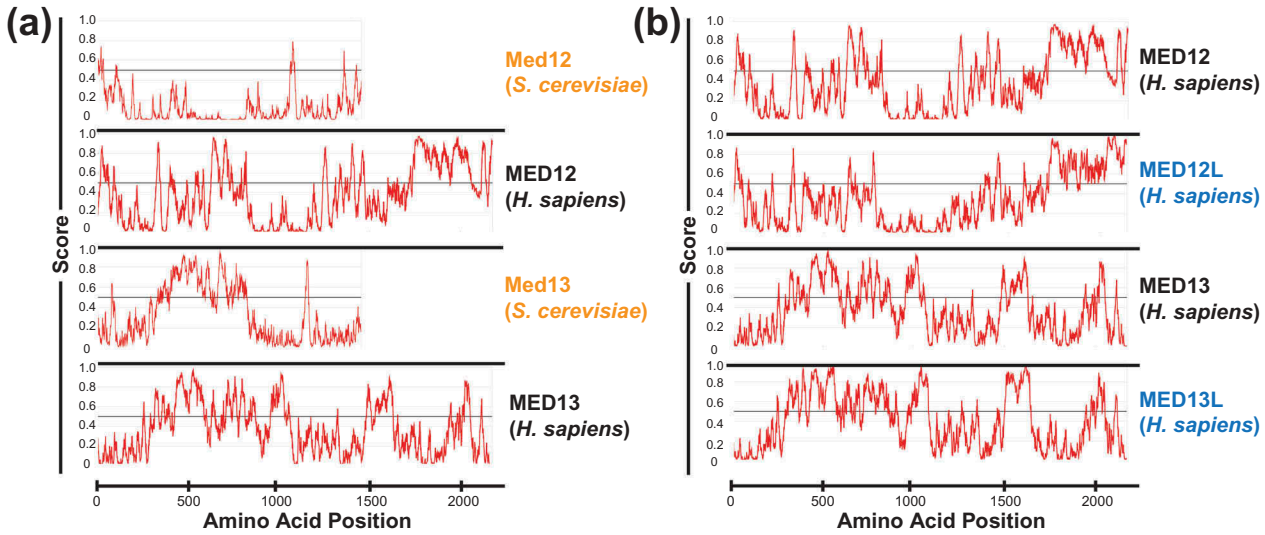


Figure 2. Summary of intrinsically disordered regions (IDRs) in kinase module subunits. **a)** Comparison of yeast (*S. cerevisiae*) Med12 and Med13 with human MED12 and MED13. Overall sequence identity is about 13% for yeast vs. human MED12 and MED13. **b)** Comparison of human MED12, MED12L and human MED13, MED13L. Not shown are CDK8, CDK19, and CCNC, which are largely structured, but each contains IDRs at their C-termini. Plots were generated with IUPred2A[152]; regions with values over 0.5 are considered disordered.

intrinsically disordered region in MED1 shows this behavior[142], and evidence in cells suggests Mediator can phase separate [141,142]. Potentially, liquid droplets that contain the CDK8 module may disperse droplets formed with pol II or the pol II CTD. Biochemical experiments indicate that Mediator–CDK8 module association is mutually exclusive with Mediator–pol II [146,147], and it is expected that this would manifest in phase separated compartments as well. Such compartmentalization may help regulate distinct stages of transcription by physically sequestering initiation factors (e.g. TFIID, Mediator, unphosphorylated pol II) from elongation factors (e.g. P-TEFb, CDK8 module, spliceosome).

Disclosure statement

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