REVIEW

Cdk7: a kinase at the core of transcription and in the crosshairs of cancer drug discovery

Robert P. Fisher

Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

ABSTRACT

The transcription cycle of RNA polymerase II (Pol II) is regulated by a set of cyclin-dependent kinases (CDKs). Cdk7, associated with the transcription initiation factor TFIIF, is both an effector CDK that phosphorylates Pol II and other targets within the transcriptional machinery, and a CDK-activating kinase (CAK) for at least one other essential CDK involved in transcription. Recent studies have illuminated Cdk7 functions that are executed throughout the Pol II transcription cycle, from promoter clearance and promoter-proximal pausing, to co-transcriptional chromatin modification in gene bodies, to mRNA 3'-end formation and termination. Cdk7 has also emerged as a target of small-molecule inhibitors that show promise in the treatment of cancer and inflammation. The challenges now are to identify the relevant targets of Cdk7 at each step of the transcription cycle, and to understand how heightened dependence on an essential CDK emerges in cancer, and might be exploited therapeutically.

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Introduction

Cdk7 was among the first CDKs to be implicated in Pol II transcription, as the kinase associated with TFIIF isolated from cell extracts, and through genetic characterization of *KIN28*, which encodes its budding yeast ortholog. In parallel, a TFIIF-free form of the trimeric Cdk7 complex (containing regulatory subunits cyclin H and Mat1), was identified as the major CAK in mammalian cell extracts, capable of phosphorylating effector CDKs on the activation segment (T loop) to promote cell-cycle progression (reviewed in [1]). The CAK function of Cdk7 and its orthologs has since been validated genetically in metazoans and fission yeast, but is not shared by budding yeast Kin28 [1–3]. This evolutionary divergence has important implications for understanding Cdk7 functions in Pol II transcription; in human cells, Cdk7 is responsible for activating Cdk9, the catalytic subunit of positive transcription elongation factor b (P-TEFb), through T-loop phosphorylation [4,5], whereas both budding and fission yeast maintain a separate CAK for activating transcriptional CDKs, including the orthologs of Cdk9 [1,6–10]. Here I review recent work that has extended and deepened our understanding of Cdk7 as a regulator of the Pol II transcription cycle and a potential target for cancer therapy.

Start at the tail: new insights into CTD phosphorylation by Cdk7

The first identified target of TFIIF-associated kinase activity was the carboxy-terminal domain (CTD) of Rpb1, the largest subunit of Pol II, which contains multiple repeats (52 in human Rpb1) of a heptad sequence (consensus: Y₁S₂P₃T₄S₅P₆S₇). Within the CTD repeats, Cdk7 preferentially phosphorylates the Ser5 and Ser7 positions, although other kinases are also likely to contribute to these modifications in vivo [11–13]. These marks predominate on Pol II transcribing the promoter-proximal and upstream regions of genes [11,14,15], suggesting that important functions of Cdk7 are executed early in the transcription cycle, consistent with its residence in TFIIF, one of the general initiation factors that assemble into the preinitiation complex (PIC) at all active Pol II promoters. Recent studies provide both genetic and biochemical support for the previously inferred roles of Cdk7 and Kin28 in dissociating Pol II from the Mediator complex that bridges the PIC and upstream regulatory elements, and in 5'-end capping of the nascent transcript [16–19]. A potentially pivotal, early function of Cdk7-dependent CTD phosphorylation was recently

revealed: the dissolution of Pol II clusters or “hubs” – phase-separated liquid droplets consisting of Pol II molecules with unphosphorylated CTDs, nucleated near the promoter by transcriptional activator proteins – to allow “entry” into the transcription cycle [20], (Figure 1). As possibly the first CDK to encounter the CTD during transcription, Cdk7 might be uniquely suited for this function. Cdk7 also has transcriptional targets apart from the CTD, and roles in the transcription cycle beyond the promoter-proximal region, which are not completely understood.

Cdk7 functions in vivo: better understanding through chemistry (and genetics)

Many insights into the functions of Cdk7, and its potential as an anti-cancer drug target, have come from chemical biology. Cdk7 and its yeast orthologs have long been prime targets for a chemical-genetic strategy: the introduction of mutations to sensitize kinases to small molecules that do not affect the wild-type versions [21]. In its simplest iteration, this involves the substitution of a conserved, bulky

residue within the ATP-binding site (the “gatekeeper”) with a less bulky residue such as Gly, to render the mutant “analog-sensitive” (AS), i.e., susceptible to inhibition by N6-substituted adenine analogs. This strategy uncovered functions of Kin28 [16,22,23]; Mcs6, the fission yeast ortholog of Cdk7 [18,24,25]; and human Cdk7 [26], which was the first kinase to be replaced by an AS mutant variant in human cancer cells [3].

More recently, Kin28 was sensitized to a new class of inhibitors, in which the adenine analog is linked to a thiol-reactive chloromethyl ketone (CMK) moiety [27]. *KIN28* required two mutations to become thus “irreversibly sensitized” (IS): the gatekeeper substitution previously used to generate *kin28^{as}*, and a Cys residue introduced at a position where it can react covalently with CMK. Treatment of a *kin28^{is}* strain with CMK uncovered an “elongation checkpoint” in transcription, possibly analogous to elongation control mechanisms in higher eukaryotes, which had evaded detection in previous studies with reversible inhibitors in *kin28^{as}* cells [27]. Coincidentally, a naturally occurring Cys residue in wild-type

Figure 1. Cdk7 functions during the Pol II transcription cycle. Cdk7 acts at multiple points during progression through the Pol II transcription cycle. These functions can be direct effects of Cdk7-mediated phosphorylation, for example, of the CTD on Ser5 and Ser7 positions (only pSer5 is shown for clarity) to dissolve Pol II clusters and allow entry into transcription. Later in the cycle, Cdk7 works through unknown targets to implement a promoter-proximal pause (dashed line) and indirectly, through activation of Cdk9/cyclin T1 (P-TEFb), to release the pause. Later events are mediated by Cdk12/and Cdk13/cyclin K, which might also be targets of the CAK function of Cdk7 (gray arrow).

human Cdk7, Cys312, is the basis for inhibition by THZ1, one of a class of recently developed covalent kinase inhibitors [4]. THZ1 has shown potent and selective cytotoxicity towards a diverse set of human cancer-derived cells, and a C312S substitution in *CDK7* conferred resistance to tumor cell killing [4,28–30]. This is essentially the AS kinase strategy in reverse – use of a *desensitizing* mutation to validate Cdk7 as a relevant drug target – and a powerful addition to the chemical-genetic toolbox. Interestingly, the *F91G* gatekeeper mutation that sensitized Cdk7 to bulky adenine analogs [3,26] also conferred resistance to THZ1 and a second covalent inhibitor, YKL-1-116, allowing reciprocal validation of *CDK7* allele-specific effects, with unrelated drugs, in isogenic pairs of *CDK7*^{WT} and *CDK7*^{as} cell lines [31].

Caps off to Cdk7 inhibition

Among eukaryotic RNA polymerases, the CTD – and the capacity for regulation by phosphorylation that it confers – are unique to Pol II. Similarly, a distinguishing feature of Pol II transcripts, not present on RNAs transcribed by Pol I or Pol III, is the 5′-monomethyl-guanosine cap added in a three-step reaction to elongating RNA chains. These two defining traits of Pol II transcription are mechanistically linked; co-transcriptional capping is far more efficient than capping of free RNA in vitro [32], and the Pol II CTD plays an essential, phosphorylation-dependent role in targeting the capping machinery to transcription complexes in vivo [33–35]. A priori, Cdk7 was a candidate to perform this coupling function because of its function early in transcription. Its preference for the Ser5 position, moreover, suggested another way Cdk7 might be specialized to promote capping: CTD-derived peptides phosphorylated on Ser5 (pSer5), but not ones phosphorylated on Ser2 (pSer2), allosterically activated the guanylyltransferase component of the mammalian capping apparatus, whereas either modification sufficed to increase the affinity of peptide binding to the capping enzyme [36].

The suspected role of the TFIIH-associated kinase Kin28 in capping of Pol II transcripts was borne out by classical and chemical genetic studies in budding yeast [16,37]. More recently, diminished capping enzyme recruitment to transcription complexes was

observed upon chemical-genetic inhibition of Cdk7^{as} in human colorectal cancer (CRC) cells [38]. In fission yeast, inactivation of Mcs6^{as} impeded another discrete step in 5′-end maturation – recruitment of the cap methyltransferase Pcm1 [18]. Interestingly, Pcm1 resides in a stoichiometric complex with Cdk9 and its cyclin partner Pch1 [9,13,39]. Recruitment of the last enzyme in the capping pathway together with a rate-limiting regulator of elongation [24] – an arrangement thus far described only in fission yeast – might be a quality control to ensure that only transcripts with fully mature cap structures are extended to full length.

The advent of selective inhibitors such as THZ1 has enabled dissection in vitro of Cdk7's role in co-transcriptional capping, through analyses of Pol II transcribing in nuclear extracts [17] or in defined systems with purified proteins [40]. In both cases, addition of THZ1 diminished CTD phosphorylation and impaired capping. In nuclear extracts, inhibition of Cdk7 also attenuated pausing in the promoter-proximal region [17], consistent with effects detected in cells treated with various Cdk7 inhibitors [4,5,41]. Pausing did not depend on capping, but the efficiency of capping was optimal on transcripts that had just emerged from the exit channel of Pol II, and decreased with increasing RNA length beyond that point, possibly consistent with capping being facilitated by Pol II pausing, to keep the 5′ end in proximity to CTD-bound capping enzymes [17], (Figure 2).

A similar length dependence was not seen in the defined system; transcripts of 23 or 223 nt were modified with equal efficiency by capping enzyme added after elongation, and capping of the longer RNA was only modestly less efficient than that of the shorter transcript when CTD phosphorylation was blocked by THZ1 [40]. This might suggest that the length dependence seen in the earlier study was imposed by a Cdk7-sensitive inhibitor of capping, which was detected on transcription complexes assembled in the nuclear extract [17], but was presumably missing from the defined system. Future studies to identify this factor might resolve the discrepancy, and help to elucidate the mechanism by which Cdk7 couples transcription and capping.

Noe Gonzalez et al. also studied co-transcriptional capping with artificial ternary complexes assembled

Figure 2. An early checkpoint in transcription. Cdk7, together with regulatory subunits cyclin H and Mat1, is part of the general transcription factor (GTF) TFIIF that assembles into the preinitiation complex (PIC) at Pol II promoters. Another GTF in the PIC, TFIIE, binds the clamp region of Pol II (top panel). Between initiation and the promoter-proximal pause, Cdk7 acts by an unknown exchange mechanism to promote displacement of TFIIE and recruitment to the Pol II clamp of DSIF, which, together with NELF, establishes the pause. Concomitant with pausing, Cdk7 phosphorylates the Pol II CTD to tether the mammalian capping enzyme (MCE) in proximity to the 5'-triphosphate end of the nascent RNA transcript (middle panel). Cdk7 is the activating kinase for Cdk9/cyclin T1 (P-TEFb), which phosphorylates DSIF (converting it to a positive elongation factor), NELF (which dissociates) and Pol II, to trigger pause release and rapid elongation of the 5'-capped transcript (bottom panel).

in vitro with DNA and RNA oligonucleotides and purified Pol II – wild-type or a mutant lacking the CTD. Removal of the CTD had no effect on capping efficiency in the absence of added TFIIF, whereas addition of TFIIF stimulated co-transcriptional

capping only in the presence of the CTD, indicating that 1) the stimulatory function of the CTD depends entirely on its phosphorylation, and 2) the only target of Cdk7 relevant for capping is the CTD. Interestingly, even in the absence of a CTD, capping

was much more efficient on RNA in the ternary complex with Pol II than on free RNA, reflecting a previously unknown interaction of Pol II with the capping apparatus that is species-specific, and both CTD- and phosphorylation-independent [40].

This system also allowed a test of whether the allosteric effect previously detected with CTD-derived phosphopeptides [36] contributed to the stimulation of co-transcriptional capping by Cdk7. Surprisingly, it didn't: CTD-derived peptides containing pSer5, added in trans to ternary transcription complexes assembled with CTD-less Pol II, did not stimulate capping, even though they did recapitulate the previously reported allosteric effect by promoting the formation of a capping enzyme-GMP intermediate [40]. Therefore, the major (and perhaps sole) function of Cdk7-mediated phosphorylation in promoting co-transcriptional capping appears to be in "tethering" the capping enzyme to the Pol II CTD, ensuring proximity to the 5'-end of the nascent transcript as it emerges from the RNA exit channel (Figure 2). Consistent with this idea, the essential requirement for pSer5 in fission yeast can be bypassed by fusion of the mammalian capping enzyme to the CTD of an Rpb1 variant in which all Ser5 positions were replaced with Ala (*rpb1-S5A-MCE1*) [35]. It is possible that allostery plays a role in vivo but can do so only in cis, in the context of a capping enzyme tethered to the transcription complex via the phosphorylated CTD.

Cdk7, giving pause (to transcription)

Another, recently uncovered function of Cdk7 is executed between initiation and the onset of rapid elongation. In metazoans, pausing by Pol II occurs in the first 50–70 nucleotides downstream of the transcriptional start site. A similar, promoter-proximal pausing event was detected in fission yeast, but not in budding yeast, by precision nuclear run-on transcription and sequencing (PRO-seq) analysis [42]. Pausing by Pol II in multicellular organisms regulates many genes important for development, cell-cycle progression and signal responses [43]. More generally, stable or transient pausing is thought to impose a checkpoint to ensure "co-transcriptionality" – the temporal coupling of RNA-processing and

chromatin modification with ongoing RNA synthesis [44]. Establishment of the pause requires an exchange of conserved initiation factors and elongation factors, which bind in mutually exclusive fashion to the clamp region of Pol II (or its archaeal orthologs) [45–49]. Release into productive elongation requires the catalytic function of P-TEFb, which phosphorylates multiple components of the paused complex to trigger its activation [50,51]. Cdk7 appears to play a dual function in pausing: first, to promote the handoff between initiation and elongation factors, needed to impose the pause; and second, to activate P-TEFb, to trigger release from the pause [5,12], (Figure 2).

The mechanism by which Cdk7 can promote pause release is self-evident – as a CAK for P-TEFb, which appears to be rate-limiting for Pol II elongation in all eukaryotes [24,52,53]. The Cdk7 requirement in Cdk9 T-loop phosphorylation, first detected in a *CDK7^{as}* CRC line treated with bulky adenine analogs [5], was recapitulated in cells derived from diverse tumor types treated with THZ1 [4,28]. The seemingly opposing role of Cdk7 in establishing the pause has likewise been detected in human cells treated with inhibitors of both AS and wild-type enzymes, by chromatin immunoprecipitation (ChIP) analyses that revealed 1) rapid attenuation of the paused peak of Pol II in the promoter-proximal region; and 2) decreased recruitment of the two principal factors needed for pausing, the DRB sensitivity-inducing factor (DSIF) and negative elongation factor (NELF) [4,5,12,41,54]. Impaired DSIF recruitment and attenuated pausing were also recapitulated in vitro, during transcription by Pol II in nuclear extracts treated with THZ1 [17]. It is unclear, however, *how* Cdk7 promotes the exchange of factors, in which DSIF and NELF displace initiation factors, including TFIIE. Both DSIF and TFIIE contain sites phosphorylated by Cdk7 in vitro [5,26,55]. A mechanistic understanding of pause establishment – and its possible regulation by these or other phosphorylations – will require reconstitution of this step in vitro, as has now been accomplished for pause release [50,51]. It is also unknown how the two opposing functions of Cdk7 in pause establishment and release are coordinated, such that Pol II can be paused more stably at repressed genes than at active ones.

The need for further molecular dissection is underscored by a recent study, using the same *CDK7^{as}* CRC line in which the Cdk7 requirement for pausing was first detected, which uncovered *increased* pausing by Pol II upon Cdk7 inhibition, and suggested that the requirement for Cdk7 in pause release might predominate genome-wide, at least after prolonged inhibitor treatments [38].

After a pause: roles of Cdk7 at later stages in transcription

The effects of Cdk7 loss of function are not restricted to the early stages of the Pol II transcription cycle; altered patterns of chromatin modification, impaired 3'-end formation and delayed termination have all been detected after inhibition of Cdk7. In interpreting these effects, however, the duality of Cdk7 function, as both an effector kinase and an upstream activator of other CDKs [1], must be kept in mind (Figure 1). An instructive example is the apparently stringent requirement for Cdk7 activity in histone H2B mono-ubiquitylation (H2Bub1), and in the H2Bub1-dependent maturation of mRNAs encoding human histones [5]. In mammalian cells, the bulk of histone gene expression arises from transcription restricted to S phase, leading to the production of non-polyadenylated mRNAs through a specialized pathway of 3'-end cleavage and termination [56]. This mechanism is dependent on both NELF [57] and Cdk9 [58], which depend on Cdk7 for their recruitment and activation, respectively [5,12]. Therefore, Cdk7 might be influencing H2Bub1 and histone mRNA 3'-end formation through its ability to regulate pause establishment or release, or through another, as-yet unidentified mechanism. Cdk7 inhibition in human cells also impairs 3'-end processing of polyadenylated mRNAs and small nuclear RNAs (snRNAs), but to a lesser extent than does inhibition of Cdk9 [5,12,54,59], perhaps consistent with an indirect effect of impaired Cdk9 function.

Likewise, effects of Cdk7 inhibition on histone modification patterns might reflect direct functions of the kinase, or secondary effects mediated by CDKs that act downstream of Cdk7. These include Cdk9, and possibly Cdk2 and Cdk13 – two closely related CDKs implicated in late events in transcription and likely to be responsible for

pSer2 *in vivo* [60,61], (Figure 1). (Both Cdk12 and Cdk13 depend on phosphorylation for full activity [62,63], and have T-loop sequences that are nearly identical to each other and to that of Cdk9, a known Cdk7 target; it is likely, but not proven, that Cdk7 is also the activating kinase for these transcriptional CDKs.) Cdk9 or its yeast orthologs have been implicated in multiple, co-transcriptional histone modification pathways, including H2Bub1 and methylation of histone H3 Lys4 (H3K4me3) and Lys 36 (H3K36me3) [58,64–67]. Inhibition of Cdk7^{as} in human cells diminished all three marks [5,38], and impeded recruitment of the H3K4 methyltransferase SETD1A/B [38]. It remains to be seen whether any of these is a direct effect of Cdk7 inhibition, rather than a consequence of impaired Cdk9 function. It is interesting in this regard that, in fission yeast, where the Cdk7 ortholog Msc6 is *not* responsible for Cdk9 activation [9], H2Bub1, H3K4me3 and H3K36me3 were all exquisitely sensitive to chemical-genetic inhibition of Cdk9, but only mildly sensitive or refractory to inhibition of Msc6 [66,68].

Cdk7, a drug target hiding in plain sight

CDKs dedicated to the control of cell proliferation have long attracted attention as potential anti-cancer drug targets [69]. Targeting CDKs in the transcription machinery, which is active in both dividing and non-dividing cells, seemed inherently more risky. Not all transcription by Pol II is equally dependent on transcriptional CDKs, however, as early studies documented in both yeast and mammalian cells [12,16,18]. A potential vulnerability of cancer cells to drugs targeting transcription emerged with the characterization of tumor-specific super-enhancers (SEs) – regulatory DNA elements that recruit large quantities of transcription machinery to drive high-level expression of genes that determine cell identity and survival [70,71]. Cdk7 is enriched in SEs, and disruption of SE-dependent transcription appears to be the basis for tumor-selective cytotoxic effects of THZ1 in T-cell leukemia (T-ALL) cells dependent on the oncogenic transcription factor RUNX1 [4], as well as in neuroblastoma [28] and small-cell lung cancer (SCLC) [29] cells dependent on MYC

isoforms. Not all transcriptional “addiction” and consequent Cdk7-dependence arises from reliance on a single oncogenic transcription factor, however, as demonstrated by the THZ1-sensitivity of triple-negative breast cancer (TNBC) cells that instead require expression of an “Achilles’ cluster” of SE-driven, TNBC-specific genes for survival [30].

The cytotoxic efficacy of THZ1 appears to correlate with dependencies on specific genes that are repressed by drug concentrations insufficient to cause global transcriptional shutoff. Even within a cancer type such as T-ALL, however, not all tumors are equally sensitive [4]. A way to expand the range of tumors susceptible to Cdk7 inhibition emerged from studies of CRC cells, in which dependency on Cdk7 was *induced* by activation of the tumor suppressor p53. Treatment with the anti-metabolite 5-fluorouracil (5-FU) or the direct p53 stabilizer nutlin-3, in combination with a Cdk7 inhibitor (a bulky adenine analog in *CDK7^{as}* cells, THZ1 or the more selective YKL-1-116 in wild-type cells), led to synergistic cell killing [31]. Apoptosis induced by these drug combinations was dependent on ongoing transcription and associated with redirection of p53 signaling; expression of p53-responsive genes implicated in cell-cycle arrest was dampened, whereas induction of certain pro-apoptotic targets was unaffected, by Cdk7 inhibition. Non-transformed cells were resistant to these drug combinations, as were cells with loss-of-function mutations in *TP53* (encoding p53), but p53-proficient melanoma- and breast cancer-derived cells were sensitive.

Therefore, the combination of Cdk7 inhibition with p53 activation is *synthetically lethal* to susceptible cancer cells, potentially providing a therapeutic strategy to 1) reduce off-target toxicity by lowering the effective doses of the component drugs, and 2) prevent emergence of drug resistance [72]. This provides a rationale for future efforts to discover additional synthetic-lethal combinations with Cdk7 inhibitors, including ones that can target tumors with *TP53* loss-of-function mutations. In addition, the selective ability of Cdk7 inhibitors to kill cancer cells with deranged gene expression [73] might provide a precedent for their use in other diseases typified by aberrant cell signaling, such as inflammation. Indeed, recent reports have indicated efficacy of a Cdk7 inhibitor in mouse models of arthritis [74,75].

Concluding remarks

Work over the past decade has yielded major advances in understanding of Cdk7’s cellular functions, while also raising the exciting prospect that it will be an important target in cancer therapy. Unanswered questions and unmet challenges remain, on both fronts. Cdk7 is itself regulated by T-loop phosphorylation, which increases when cells are stimulated to enter the division cycle [76] but preferentially affects kinase activity towards transcriptional substrates, including the CTD [5,26,77]. Does this mitogen-responsive modification – by an as-yet unidentified kinase – regulate expression of genes needed for proliferation? Another, newly arisen mechanistic question is whether Cdk7 is exclusively responsible for dissolving Pol II clusters [20], or if other CTD kinases can perform this function to facilitate transcription. Similarly, can other transcriptional CDKs be targeted in cancer therapy, as recent results with a Cdk12/13 inhibitor seem to suggest [78], and will tumors be differentially susceptible to drugs (or drug combinations) targeting different CDKs? With improved biochemical and structural insights into transcription complexes at various stages of the Pol II cycle, and with powerful new chemical, genetic *and chemical-genetic* tools, the answers to these questions (and others) are very likely to emerge over the next decade.

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References

- [1] Fisher RP. Secrets of a double agent: CDK7 in cell-cycle control and transcription. *J Cell Sci.* 2005;118:5171–5180.

- [2] Ganuza M, Saiz-Ladera C, Canamero M, et al. Genetic inactivation of Cdk7 leads to cell cycle arrest and induces premature aging due to adult stem cell exhaustion. *Embo J.* **2012**;31:2498–2510.
- [3] Larochelle S, Merrick KA, Terret ME, et al. Requirements for Cdk7 in the assembly of Cdk1/cyclin B and activation of Cdk2 revealed by chemical genetics in human cells. *Mol Cell.* **2007**;25:839–850.
- [4] Kwiatkowski N, Zhang T, Rahl PB, et al. Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. *Nature.* **2014**;511:616–620.
- [5] Larochelle S, Amat R, Glover-Cutter K, et al. Cyclin-dependent kinase control of the initiation-to-elongation switch of RNA polymerase II. *Nat Struct Mol Biol.* **2012**;19:1108–1115.
- [6] Espinoza FHE, Farrell A, Nourse JL, et al. Cak1 is required for Kin28 phosphorylation and activation *in vivo*. *Mol Cell Biol.* **1998**;18:6365–6373.
- [7] Kimmelman J, Kaldis P, Hengartner CJ, et al. Activating phosphorylation of the kin28p subunit of yeast TFIIF by cak1p. *Mol Cell Biol.* **1999**;19:4774–4787.
- [8] Ostapenko D, Solomon MJ. Phosphorylation by Cak1 regulates the C-terminal domain kinase Ctk1 in *Saccharomyces cerevisiae*. *Mol Cell Biol.* **2005**;25:3906–3913.
- [9] Pei Y, Du H, Singer J, et al. Cyclin-dependent kinase 9 (Cdk9) of fission yeast is activated by the CDK-activating kinase Csk1, overlaps functionally with the TFIIF-associated kinase Mcs6, and associates with the mRNA cap methyltransferase Pcm1 *in vivo*. *Mol Cell Biol.* **2006**;26:777–788.
- [10] Yao S, Prelich G. Activation of the Bur1-Bur2 cyclin-dependent kinase complex by Cak1. *Mol Cell Biol.* **2002**;22:6750–6758.
- [11] Akhtar MS, Heidemann M, Tietjen JR, et al. TFIIF kinase places bivalent marks on the carboxy-terminal domain of RNA polymerase II. *Mol Cell.* **2009**;34:387–393.
- [12] Glover-Cutter K, Larochelle S, Erickson B, et al. TFIIF-associated Cdk7 kinase functions in phosphorylation of C-terminal domain Ser7 residues, promoter-proximal pausing, and termination by RNA polymerase II. *Mol Cell Biol.* **2009**;29:5455–5464.
- [13] St Amour CV, Sanso M, Bosken CA, et al. Separate domains of fission yeast Cdk9 (P-TEFb) are required for capping enzyme recruitment and primed (Ser7-Phosphorylated) Rpb1 Carboxyl-terminal domain substrate recognition. *Mol Cell Biol.* **2012**;32:2372–2383.
- [14] Bataille AR, Jeronimo C, Jacques PE, et al. A universal RNA polymerase II CTD cycle is orchestrated by complex interplays between kinase, phosphatase, and isomerase enzymes along genes. *Mol Cell.* **2012**;45:158–170.
- [15] Tietjen JR, Zhang DW, Rodriguez-Molina JB, et al. Chemical-genomic dissection of the CTD code. *Nat Struct Mol Biol.* **2010**;17:1154–1161.
- [16] Kanin EI, Kipp RT, Kung C, et al. Chemical inhibition of the TFIIF-associated kinase Cdk7/Kin28 does not impair global mRNA synthesis. *Proc Natl Acad Sci U S A.* **2007**;104:5812–5817.
- [17] Nilson KA, Guo J, Turek ME, et al. THZ1 reveals roles for Cdk7 in co-transcriptional capping and pausing. *Mol Cell.* **2015**;59:576–587.
- [18] Viladevall L, St Amour CV, Rosebrock A, et al. TFIIF and P-TEFb coordinate transcription with capping enzyme recruitment at specific genes in fission yeast. *Mol Cell.* **2009**;33:738–751.
- [19] Wong KH, Jin Y, Struhl K. TFIIF phosphorylation of the Pol II CTD stimulates mediator dissociation from the preinitiation complex and promoter escape. *Mol Cell.* **2014**;54:601–612.
- [20] Boehning M, Dugast-Darzacq C, Rankovic M, et al. RNA polymerase II clustering through carboxy-terminal domain phase separation. *Nat Struct Mol Biol.* **2018**;25:833–840.
- [21] Knight ZA, Shokat KM. Chemical genetics: where genetics and pharmacology meet. *Cell.* **2007**;128:425–430.
- [22] Hong SW, Hong SM, Yoo JW, et al. Phosphorylation of the RNA polymerase II C-terminal domain by TFIIF kinase is not essential for transcription of *Saccharomyces cerevisiae* genome. *Proc Natl Acad Sci U S A.* **2009**;106:14276–14280.
- [23] Liu Y, Kung C, Fishburn J, et al. Two cyclin-dependent kinases promote RNA polymerase II transcription and formation of the scaffold complex. *Mol Cell Biol.* **2004**;24:1721–1735.
- [24] Booth GT, Parua PK, Sanso M, et al. Cdk9 regulates a promoter-proximal checkpoint to modulate RNA polymerase II elongation rate in fission yeast. *Nat Commun.* **2018**;9:543.
- [25] Devos M, Mommaerts E, Migeot V, et al. Fission yeast Cdk7 controls gene expression through both its CAK and C-terminal domain kinase activities. *Mol Cell Biol.* **2015**;35:1480–1490.
- [26] Larochelle S, Batliner J, Gamble MJ, et al. Dichotomous but stringent substrate selection by the dual-function Cdk7 complex revealed by chemical genetics. *Nat Struct Mol Biol.* **2006**;13:55–62.
- [27] Rodriguez-Molina JB, Tseng SC, Simonett SP, et al. Engineered covalent inactivation of TFIIF-kinase reveals an elongation checkpoint and results in widespread mRNA stabilization. *Mol Cell.* **2016**;63:433–444.
- [28] Chipumuro E, Marco E, Christensen CL, et al. CDK7 inhibition suppresses super-enhancer-linked oncogenic transcription in MYCN-driven cancer. *Cell.* **2014**;159:1126–1139.
- [29] Christensen CL, Kwiatkowski N, Abraham BJ, et al. Targeting transcriptional addictions in small cell lung cancer with a covalent CDK7 inhibitor. *Cancer Cell.* **2014**;26:909–922.
- [30] Wang Y, Zhang T, Kwiatkowski N, et al. CDK7-dependent transcriptional addiction in triple-negative breast cancer. *Cell.* **2015**;163:174–186.

- [31] Kalan S, Amat R, Schachter MM, et al. Activation of the p53 transcriptional program sensitizes cancer cells to Cdk7 inhibitors. *Cell Rep.* **2017**;21:467–481.
- [32] Moteki S, Price D. Functional coupling of capping and transcription of mRNA. *Mol Cell.* **2002**;10:599–609.
- [33] Cho EJ, Takagi T, Moore CR, et al. mRNA capping enzyme is recruited to the transcription complex by phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes Dev.* **1997**;11:3319–3326.
- [34] McCracken S, Fong N, Rosonina E, et al. 5'-Capping enzymes are targeted to pre-mRNA by binding to the phosphorylated carboxy-terminal domain of RNA polymerase II. *Genes Dev.* **1997**;11:3306–3318.
- [35] Schwer B, Shuman S. Deciphering the RNA polymerase II CTD code in fission yeast. *Mol Cell.* **2011**;43:311–318.
- [36] Ho CK, Shuman S. Distinct roles for CTD Ser-2 and Ser-5 phosphorylation in the recruitment and allosteric activation of mammalian mRNA capping enzyme. *Mol Cell.* **1999**;3:405–411.
- [37] Schroeder SC, Schwer B, Shuman S, et al. Dynamic association of capping enzymes with transcribing RNA polymerase II. *Genes Dev.* **2000**;14:2435–2440.
- [38] Ebmeier CC, Erickson B, Allen BL, et al. Human TFIIF kinase CDK7 regulates transcription-associated chromatin modifications. *Cell Rep.* **2017**;20:1173–1186.
- [39] Guiguen A, Soutourina J, Dewez M, et al. Recruitment of P-TEFb (Cdk9-Pch1) to chromatin by the cap-methyl transferase Pcm1 in fission yeast. *Embo J.* **2007**;26:1552–1559.
- [40] Noe Gonzalez M, Sato S, Tomomori-Sato C, et al. CTD-dependent and -independent mechanisms govern co-transcriptional capping of Pol II transcripts. *Nat Commun.* **2018**;9:3392.
- [41] Kelso TW, Baumgart K, Eickhoff J, et al. Cyclin-dependent kinase 7 controls mRNA synthesis by affecting stability of preinitiation complexes, leading to altered gene expression, cell cycle progression, and survival of tumor cells. *Mol Cell Biol.* **2014**;34:3675–3688.
- [42] Booth GT, Wang IX, Cheung VG, et al. Divergence of a conserved elongation factor and transcription regulation in budding and fission yeast. *Genome Res.* **2016**;26:799–811.
- [43] Adelman K, Lis JT. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat Rev Genet.* **2012**;13:720–731.
- [44] Perales R, Bentley D. “Cotranscriptionality”: the transcription elongation complex as a nexus for nuclear transactions. *Mol Cell.* **2009**;36:178–191.
- [45] Bernecky C, Plitzko JM, Cramer P. Structure of a transcribing RNA polymerase II-DSIF complex reveals a multidentate DNA-RNA clamp. *Nat Struct Mol Biol.* **2017**;24:809–815.
- [46] Ehara H, Yokoyama T, Shigematsu H, et al. Structure of the complete elongation complex of RNA polymerase II with basal factors. *Science.* **2017**;357:921–924.
- [47] Grohmann D, Nagy J, Chakraborty A, et al. The initiation factor tfe and the elongation factor Spt4/5 compete for the RNAP clamp during transcription initiation and elongation. *Mol Cell.* **2011**;43:263–274.
- [48] Klein BJ, Bose D, Baker KJ, et al. RNA polymerase and transcription elongation factor Spt4/5 complex structure. *Proc Natl Acad Sci U S A.* **2011**;108:546–550.
- [49] Martinez-Rucobo FW, Sainsbury S, Cheung AC, et al. Architecture of the RNA polymerase-Spt4/5 complex and basis of universal transcription processivity. *Embo J.* **2011**;30:1302–1310.
- [50] Vos SM, Farnung L, Boehning M, et al. Structure of activated transcription complex Pol II-DSIF-PAF-SPT 6. *Nature.* **2018**;560:607–612.
- [51] Vos SM, Farnung L, Urlaub H, et al. Structure of paused transcription complex Pol II-DSIF-NELF. *Nature.* **2018**;560:601–606.
- [52] Jonkers I, Kwak H, Lis JT. Genome-wide dynamics of Pol II elongation and its interplay with promoter proximal pausing, chromatin, and exons. *Elife.* **2014**;3:e02407.
- [53] Peterlin BM, Price DH. Controlling the elongation phase of transcription with P-TEFb. *Mol Cell.* **2006**;23:297–305.
- [54] Sanso M, Levin RS, Lipp JJ, et al. P-TEFb regulation of transcription termination factor Xrn2 revealed by a chemical genetic screen for Cdk9 substrates. *Genes Dev.* **2016**;30:117–131.
- [55] Ohkuma Y, Roeder RG. Regulation of TFIIF ATPase and kinase activities by TFIIE during active initiation complex formation. *Nature.* **1994**;368:160–163.
- [56] Dominski Z, Marzluff WF. Formation of the 3' end of histone mRNA: getting closer to the end. *Gene.* **2007**;396:373–390.
- [57] Narita T, Yung TM, Yamamoto J, et al. NELF interacts with CBC and participates in 3' end processing of replication-dependent histone mRNAs. *Mol Cell.* **2007**;26:349–365.
- [58] Pirngruber J, Shchebet A, Schreiber L, et al. CDK9 directs H2B monoubiquitination and controls replication-dependent histone mRNA 3'-end processing. *EMBO Rep.* **2009**;10:894–900.
- [59] Laitem C, Zaborowska J, Isa NF, et al. CDK9 inhibitors define elongation checkpoints at both ends of RNA polymerase II-transcribed genes. *Nat Struct Mol Biol.* **2015**;22:396–403.
- [60] Bartkowiak B, Liu P, Phatnani HP, et al. CDK12 is a transcription elongation-associated CTD kinase, the metazoan ortholog of yeast Ctk1. *Genes Dev.* **2010**;24:2303–2316.
- [61] Davidson L, Muniz L, West S. 3' end formation of pre-mRNA and phosphorylation of Ser2 on the RNA polymerase II CTD are reciprocally coupled in human cells. *Genes Dev.* **2014**;28:342–356.
- [62] Bosken CA, Farnung L, Hintermair C, et al. The structure and substrate specificity of human Cdk12/Cyclin K. *Nat Commun.* **2014**;5:3505.

- [63] Greifenberg AK, Honig D, Pilarova K, et al. Structural and functional analysis of the Cdk13/Cyclin K complex. *Cell Rep.* **2016**;14:320–331.
- [64] Chu Y, Sutton A, Sternglanz R, et al. The BUR1 cyclin-dependent protein kinase is required for the normal pattern of histone methylation by SET2. *Mol Cell Biol.* **2006**;26:3029–3038.
- [65] Laribee RN, Krogan NJ, Xiao T, et al. BUR kinase selectively regulates H3 K4 trimethylation and H2B ubiquitylation through recruitment of the PAF elongation complex. *Curr Biol.* **2005**;15:1487–1493.
- [66] Sansó M, Lee KM, Viladevall L, et al. A positive feedback loop links opposing functions of P-TEFb/Cdk9 and histone H2B ubiquitylation to regulate transcript elongation in fission yeast. *PLoS Genet.* **2012**;8:e1002822.
- [67] Wood A, Schneider J, Dover J, et al. The Bur1/Bur2 complex is required for histone H2B monoubiquitination by Rad6/Bre1 and histone methylation by COMPASS. *Mol Cell.* **2005**;20:589–599.
- [68] Mbogning J, Page V, Burston J, et al. Functional interaction of Rpb1 and Spt5 C-terminal domains in co-transcriptional histone modification. *Nucleic Acids Res.* **2015**.
- [69] Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer.* **2009**;9:153–166.
- [70] Hnisz D, Abraham BJ, Lee TI, et al. Super-enhancers in the control of cell identity and disease. *Cell.* **2013**;155:934–947.
- [71] Loven J, Hoke HA, Lin CY, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell.* **2013**;153:320–334.
- [72] O’Neil NJ, Bailey ML, Hieter P. Synthetic lethality and cancer. *Nat Rev Genet.* **2017**;18:613–623.
- [73] Cao K, Shilatifard A. Inhibit globally, act locally: CDK7 inhibitors in cancer therapy. *Cancer Cell.* **2014**;26:158–159.
- [74] Hong H, Zeng Y, Jian W, et al. CDK7 inhibition suppresses rheumatoid arthritis inflammation via blockage of NF-kappaB activation and IL-1beta/IL-6 secretion. *J Cell Mol Med.* **2018**;22:1292–1301.
- [75] Xia Y, Lin LY, Liu ML, et al. Selective inhibition of CDK7 ameliorates experimental arthritis in mice. *Clin Exp Med.* **2015**;15:269–275.
- [76] Schachter MM, Merrick KA, Larochelle S, et al. A cdk7-cdk4 T-loop phosphorylation cascade promotes g1 progression. *Mol Cell.* **2013**;50:250–260.
- [77] Larochelle S, Chen J, Knights R, et al. T-loop phosphorylation stabilizes the CDK7-cyclin H-MAT1 complex *in vivo* and regulates its CTD kinase activity. *Embo J.* **2001**;20:3749–3759.
- [78] Zhang T, Kwiatkowski N, Olson CM, et al. Covalent targeting of remote cysteine residues to develop CDK12 and CDK13 inhibitors. *Nat Chem Biol.* **2016**;12:876–884.