Protein phosphatases in the RNAPII transcription cycle: erasers, sculptors, gatekeepers, and potential drug targets

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The transcription cycle of RNA polymerase II (RNAPII) is governed at multiple points by opposing actions of cyclin-dependent kinases (CDKs) and protein phosphatases, in a process with similarities to the cell division cycle. While important roles of the kinases have been established, phosphatases have emerged more slowly as key players in transcription, and large gaps remain in understanding of their precise functions and targets. Much of the earlier work focused on the roles and regulation of sui generis and often atypical phosphatases—FCP1, Rtr1/RPAP2, and SSU72—with seemingly dedicated functions in RNAPII transcription. Decisive roles in the transcription cycle have now been uncovered for members of the major phosphoprotein phosphatase (PPP) family, including PP1, PP2A, and PP4—abundant enzymes with pleiotropic roles in cellular signaling pathways. These phosphatases appear to act principally at the transitions between transcription cycle phases, ensuring fine control of elongation and termination. Much is still unknown, however, about the division of labor among the PPP family members, and their possible regulation by or of the transcriptional kinases. CDKs active in transcription have recently drawn attention as potential therapeutic targets in cancer and other diseases, raising the prospect that the phosphatases might also present opportunities for new drug development. Here we review the current knowledge and outstanding questions about phosphatases in the context of the RNAPII transcription cycle.

Transcription in eukaryotes relies on RNA polymerases I, II, and III to synthesize different types of RNA molecules encoded in the nuclear genome. RNA polymerase II (RNAPII) transcribes the majority of genes, including those encoding proteins, long noncoding RNAs (lncRNAs), microRNAs (miRNAs), enhancer RNAs (eRNAs), and most small nuclear RNAs (snRNAs) (Hsin and Manley 2012; Cramer 2019a). Transcription by RNAPII can be modeled as a cycle comprising discrete stages of initiation, elongation, 3′ end formation and termination, and, finally, recycling of RNAPII to restart the process (Fig. 1). Transcription is strictly regulated, as befitting its critical role in determining levels of gene expression and, thus, virtually all aspects of cellular identity, fate, and metabolism. Initiation was long considered the major, rate-limiting step in transcriptional regulation—a paradigm first established in prokaryotic systems and seemingly validated by the large numbers and important roles of transcriptional activators that help recruit RNA-PII to eukaryotic promoters. A more complex picture has now emerged, in which elongation, termination, and recycling are also subject to regulation, which in many cases is determinative of transcriptional outputs (Sansó and Fisher 2013; Cramer 2019b).

The control of transcription initiation by RNAPII generally involves the regulation of DNA accessibility by chromatin-modifying and remodeling enzymes, binding to exposed regulatory elements by sequence-specific transcriptional activators, engagement of a multisubunit coactivator complex called the Mediator, and the stepwise association of general transcription factors (GTFs) and RNAPII to assemble a competent preinitiation complex (PIC) at the transcription start site (TSS) (Cramer 2019b; Schier and Taatjes 2020). In budding yeast, initiation is generally rate-limiting for the productive transcription of protein-coding genes. At a majority of genes in metazoans, however, transcription by RNAPII, once initiated, is paused ~50–100 nt downstream from the TSS and requires additional signaling to resume processive elongation (Adelman and Lis 2012; Core and Adelman 2019). Both
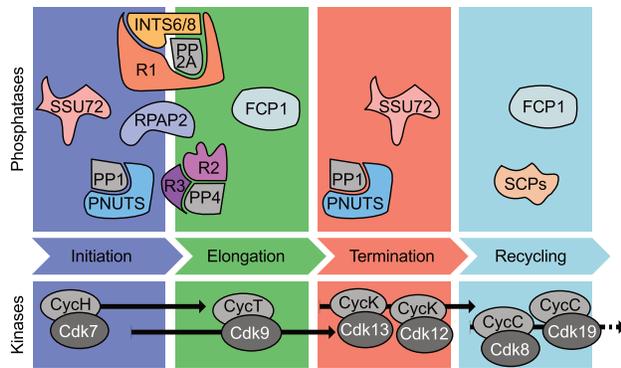
Figure 1. Protein phosphatases and kinases involved in the RNAPII transcription cycle. The protein phosphatases and cyclin-dependent kinases (CDKs) with their cognate cyclins (Cyc) associated with the sequential stages of the RNAPII transcription cycle are depicted. Horizontal arrows indicate specific intervals in which individual cyclin/CDKs act; analogous assignments of phosphatases remain imprecise. For PPPs, catalytic subunits are depicted in gray and regulatory subunits in colors.

modes of transcription by RNAPII—initiation-limited and pause-regulated—are coordinated by the phosphorylation of RNAPII and its accessory proteins.

Protein phosphorylation is the reversible, covalent addition of a phosphate group to side chains of phospho-acceptor amino acid residues—predominantly serine, threonine, or tyrosine in eukaryotes. Phosphorylation at specific residues can alter the function of a protein by directly (i.e., electrostatically) promoting or disrupting intermolecular interactions or by allosterically altering conformation of the modified protein and thereby influencing its activity, positively or negatively (Beltrao et al. 2013). High-throughput, mass spectrometric analyses mapped nearly 300,000 phosphorylation sites in the human proteome, whereas ubiquitylation and acetylation—the second and third most common post-translational modifications (PTMs) readily detectable by mass spectrometry—occur at ~110,000 and ~40,000 sites, respectively (Hornbeck et al. 2015). Protein phosphorylation is also perhaps the most ancient PTM, ubiquitous throughout evolution and in all kingdoms of life, and is believed to have played an important role in the first living organisms (Beltrao et al. 2013).

Phosphorylation is dynamic and, like other PTMs, is controlled by the coordinated action of "writers" and "erasers"—the protein kinases and phosphatases, respectively. For reasons we discuss below, the kinases that place phosphorylation marks have received more attention than the phosphatases that remove them, but both classes of enzymes are, obviously, equally important (Gelens et al. 2018). Pathways regulated by phosphorylation need phosphatase activity to maintain signaling proteins in their unmodified state in the absence of the appropriate signal and to reset the system after activation so that it can respond again when restimulated. Moreover, because signaling proteins are commonly regulated by multisite phosphorylation, with readouts that depend on precise, combinatorial modification patterns, phosphory-

lation and dephosphorylation must be executed with similar levels of precision (Smoly et al. 2017).

Cotranscriptional phosphorylation of RNAPII and its accessory factors is predominantly catalyzed by the cyclin-dependent kinases (CDKs) (for a recent review, see Parua and Fisher 2020). In a current model, different CDKs are recruited or activated in a stage-specific manner to phosphorylate substrates in, or associated with, RNAPII transcription complexes (Fig. 1). CDK-dependent phosphorylation generally acts after initiation to promote transitions between phases of the transcription cycle and to coordinate these transitions with cotranscriptional events such as RNA-processing and chromatin modification (Buratowski 2009; Hsin and Manley 2012; Sansó and Fisher 2013; Cramer 2019b). This is in many ways analogous to the cell division cycle, another process driven largely by CDK activity (Swaffer et al. 2016; Örd and Loog 2019). As discussed below, this resemblance goes beyond broad similarities of regulatory logic and includes examples of entire signaling modules being duplicated or repurposed in cell division and transcriptional machineries (Malumbres 2014).

Although the functions and relevant protein substrates of CDKs in transcription are still being deciphered, work in diverse systems has provided a robust framework for ongoing investigations. Importantly, the temporal sequence in which different CDKs act during the RNAPII cycle appears to be conserved in evolution, so that we have a good idea of which steps in transcription are affected when one or another CDK is disabled (Parua and Fisher 2020). There is less clarity regarding the phosphatases (Fig. 1). This is largely due to impediments to the study of dephosphorylating enzymes, which we discuss below. Over the last several years, however, there has been renewed interest in the transcriptional phosphatases, with concomitant development of new tools for their genetic and biochemical analysis. Here we review insights revealed by these studies, from which a more complete picture is beginning to emerge of kinases and phosphatases working in concert.

The biology of protein phosphatases

Protein kinases are among the most intensely studied enzyme classes, owing to their near ubiquity in cellular signaling pathways and their potential for therapeutic targeting in diseases including cancer. Phosphatases help determine the balance between phosphorylation and dephosphorylation of myriad proteins in vivo but are relatively refractory to many of the genetic and biochemical tools used to dissect and characterize the kinome. Much of this difficulty stems from peculiarities and complexities of phosphatase biology and regulation, for which a specialized toolkit is still being developed.

Kinases vs. phosphatases: a contrast in regulatory style

Functional diversity among protein kinases has evolved mainly through iterative gene duplication and

specialization of kinase catalytic subunits, such that genetic ablation or chemical inhibition of any one member typically affects relatively few signaling pathways in vivo (although there are important exceptions to this generalization). It has been possible, moreover, to achieve some measure of kinase selectivity with active site-directed small-molecule inhibitors that discriminate among subtle variants of the ATP-binding pocket and surrounding surface features (Wu et al. 2015; Parua and Fisher 2020). In contrast, genes encoding catalytic subunits of phosphatases are relatively few in number but are heavily transcribed and translated to maintain an overall abundance similar to that of kinases (Gelens et al. 2018). Moreover, the catalytic subunits of many phosphatases have little intrinsic substrate selectivity and are instead directed to dephosphorylate specific substrates and eschew others by interacting with accessory proteins. Although kinases can also be targeted to specific substrates or subcellular locations by binding to regulatory subunits such as cyclins, the catalytic subunits themselves typically have strong preferences for conserved motifs within their substrates. This difference has spawned a misconception that protein phosphatases are promiscuous in comparison to kinases; on the contrary, phosphatases can achieve exquisite substrate specificity in vivo through their quaternary interactions with regulatory partners (Virshup and Shenolikar 2009). Relative to kinases, however, it has been more difficult to identify protein substrates of specific phosphatases or to reconstitute and characterize the selective phosphatase holoenzymes that exist in cells.

The human genome encodes ~500 kinases (Manning et al. 2002) and 189 protein phosphatases (Chen et al. 2017), but ~90% of cellular dephosphorylation events are catalyzed by the phosphoprotein phosphatase (PPP) subclass, composed of 13 pSer/pThr phosphatases including the broadly active PP1 and PP2A (Fig. 2; Heroes et al. 2013). Consistent with its heavy workload in cells, PP1 is an abundant protein, with a cellular concentration of ~0.2 µM (Verbinnen et al. 2017). PP2A is similarly abundant, reportedly accounting for up to 1% of total cellular protein and the majority of pSer/pThr phosphatase activity in some tissues (Shi 2009; Sangodkar et al. 2016). Strikingly, the abundance of PPPs is typically orders of magnitude higher than those of transcription-related, non-PPP phosphatases, as determined by proteomic analyses (Fig. 2). In aggregate, the intracellular abundance of phosphatases has been estimated to be twice that of kinases (Smoly et al. 2017). The disproportionate numbers of kinase and phosphatase isoforms are evolutionarily conserved: The genome of the budding yeast *Saccharomyces cerevisiae* encodes 129 kinase catalytic subunits but only 30 phosphatases, of which 13 are the PPPs (Breitkreutz et al. 2010; Chen et al. 2017). Similar numbers have been reported in fission yeast, mouse, fly, and plant systems (Morrison et al. 2000; Schweighofer and Meskiene 2015; Smoly et al. 2017).

Kinases and phosphatases differ in other important ways. Whereas kinases often undergo large fluctuations in expression and activity in response to stimuli, phosphatase holoenzymes, with some exceptions, tend to be stable in vivo and more constant in their specific activity. This may reflect the canonical roles of dephosphorylation in kinase-dependent signaling: setting thresholds for initial pathway activation, ensuring timely shutoff to prevent tonic activation, and buffering noise by suppressing activation in response to weak or transient stimuli (Ceulemans and Bollen 2004; Smoly et al. 2017).

How phosphatases achieve specificity

PPPs attain a target specificity comparable to that of kinases by acting as obligate heteromers in vivo (Fig. 3). For example, despite its abundance, PP1 is probably never present in the cell as a monomer (Virshup and Shenolikar 2009); physiologically relevant, active PP1 holoenzymes are composed of catalytic and regulatory subunits. In vivo, relatively few PP1 catalytic subunits achieve functional diversity by combining with a multitude of regulators that confer distinct localizations and substrate specificities (Bertolotti 2018).

PP1 holoenzymes are composed of a catalytic subunit encoded in humans by one of three paralogous genes, *PPP1CA*, *PPP1CB*, and *PPP1CC* (encoding PP1α, PP1β, and PP1γ, respectively), with a large exposed surface able to bind one or more PP1-interacting proteins (PIPs), giving rise to both dimeric and higher-order complexes (Fig. 3). Identified PIPs currently number >200 and are relatively divergent, apart from short motifs that mediate binding to the catalytic subunit. Two such motifs contain the consensus sequences RVxF and S/GILK, both found in a large number of PIPs (Moorhead et al. 2008; Verbinnen et al. 2017). In contrast, PP2A holoenzymes are composed of a core heterodimer, including one catalytic ("C") subunit encoded by the paralogous *PPP2CA* and *PPP2CB* genes and one "A" scaffold subunit (also known as PR65 and encoded by the *PPP2R1A* and *PPP2R1B* genes), to which a third regulatory partner, the B subunit, can bind to generate functional diversity (Fig. 3; Kauko et al. 2020). Compared with PIPs, PP2A B subunits are more structurally related and can be clustered in four families (B/B55, B′/B56, B″, or B‴). B subunits are also fewer in number (two to five per family), potentially giving rise to ~80 canonical PP2A holoenzymes (Sangodkar et al. 2016). PP2B, PP4, and PP6 share a similar assembly strategy, although the known interactors for these phosphatases are fewer. Other phosphatases covered in this review belong to classes distinct from the PPPs and function as monomers (Fig. 3). FCP1 (encoded in humans by the *CTDP1* gene) and the related small CTD phosphatases (SCPs) were founding members of a new class of enzymes belonging to a broader superfamily of phosphotransferases and phosphohydrolases (Kamenski et al. 2004). SSU72 and Rtr1 (and its human ortholog RPAP2) also have active sites that are unique among pSer/pThr phosphatases (Meinhart et al. 2003; Irani et al. 2016). Detailed descriptions of their modes of action or catalytic mechanisms can be found elsewhere (Pons et al. 2014; Mayfield et al. 2016).
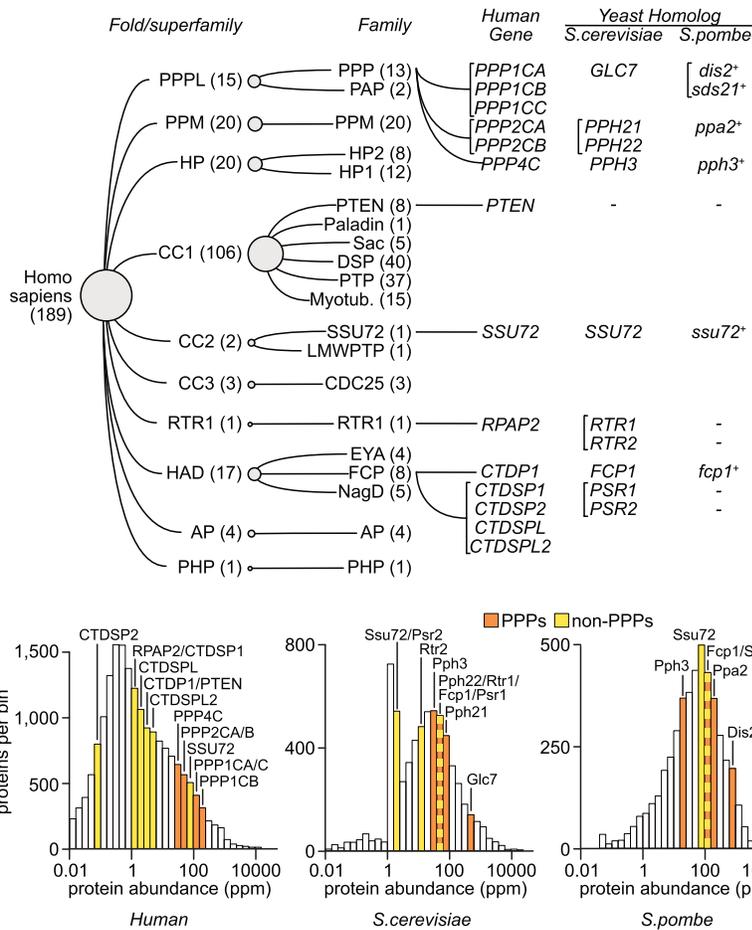
Figure 2. Human and yeast protein phosphatases implicated in RNAPII transcription. (*Top*) Hierarchical classification of 189 described human protein phosphatases (Chen et al. 2017). The "human gene" column lists genes encoding phosphatases involved in RNAPII transcription. For these genes, the corresponding homologs in *S. cerevisiae* or *S. pombe* are reported. For PPPs, only genes encoding catalytic subunits are included. In parentheses we list the numbers of phosphatases belonging to the respective human family or superfamily. In brackets we group paralogous genes. Layout is adapted from http://phosphatome.net. Homologous genes in different species are reported according to Homologene (http://ncbi.nlm.nih.gov/homologene) or Chen et al. (2017). (*Bottom*) Phosphoprotein phosphatases (PPPs) are typically more abundant than non-PPPs in human and yeast proteomes. Proteins are grouped in bins based on their abundance in the proteome. Bins containing transcription-related phosphatases (see *top* panel) are colored based on whether they are PPPs or not. Proteomic data were obtained and histogram layout was adapted from http://pax-db.org. (ppm) Parts per million.

Specialized phosphatases help drive RNAPII transcription

The importance of protein phosphorylation in governing the RNAPII transcription cycle first became apparent with the identification and characterization of the C-terminal domain (CTD) of RPB1, the largest subunit of RNAPII (encoded by the *POLR2A* gene in humans). The RPB1-CTD is composed of variable numbers of heptad repeats (52 in humans, 26 in *S. cerevisiae*, 29 in *S. pombe*) of the consensus sequence Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 (Corden 1990). Most or all residues in the CTD are subject to PTM—most commonly phosphorylation but also glycosylation, prolyl isomerization, and, in nonconsensus repeats containing Lys or Arg substitutions at position 7, methylation (Sims et al. 2011)—in stereotypical patterns correlated with transcription cycle stage (Fig. 4). These patterns have been proposed to constitute a "CTD code" that is read by RNAPII-interacting factors to coordinate transcription with cotranscriptional events (Buratowski 2003; Egloff and Murphy 2008; Schwer and Shuman 2011). There are examples in which a specific mark ("letter") or combination of marks ("word") is known to elicit a particular downstream effect, but CTD lexicography remains a work in progress. The most
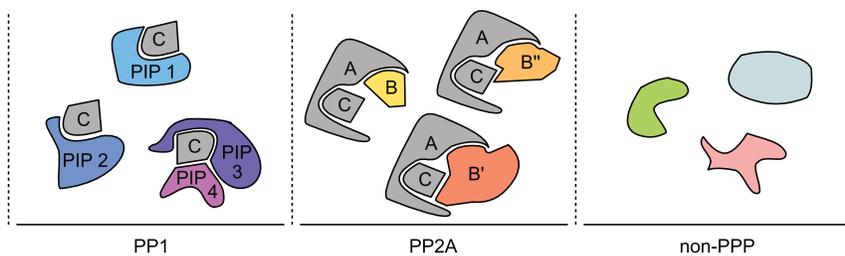
Figure 3. PPPs act as obligate heteromers. In order to specifically catalyze a large number of reactions, the catalytic subunit of a PPP ("C") must interact with one or more regulatory subunits. C subunits are encoded by paralogous genes and therefore are depicted as invariable (gray). PP1 C subunits can interact with a large range of PP1-interacting proteins (PIPs) in both heterodimeric and higher-order complexes. In canonical PP2A holoenzymes, C subunits always interact with one scaffold A subunit (encoded by two similar genes, also depicted as invariable; gray) and one B subunit (encoded by multiple genes). Non-PPPs generally act as monomers.

extensively investigated modifications are phosphorylations of the Ser5 (pSer5) and Ser2 (pSer2) positions, which predominate in RNAPII complexes sampled early and late in transcription, respectively (Fig. 4).

Multiple kinases, including a subset of the CDK family comprising at least seven members in humans, have been implicated in CTD phosphorylation (Parua and Fisher 2020). It is now clear that many of these kinases phosphorylate components of the RNAPII machinery besides the CTD and can thus influence the transcription cycle through diverse mechanisms beyond recruitment of factors to the RNAPII complex (Larochelle et al. 2006; Poss et al. 2016; Sansó et al. 2016; Rimel et al. 2020). For example, the major substrate of positive transcription elongation factor b (P-TEFb, a complex of CDK9 and cyclin T1) is SPT5, the essential subunit of the conserved SPT4/SPT5 complex also known in metazoans as DRB sensitivity-inducing factor (DSIF). CDK9 phosphorylates SPT5 on multiple sites (Fig. 4) to regulate RNAPII pause release and processive elongation, as described in more detail below (Yamada et al. 2006; Booth et al. 2018; Vos et al. 2018a, b). Similarly, while many of the phosphatases that act during transcription were first identified by virtue of activity toward the RPB1-CTD, new targets and functions of these enzymes are emerging. Self-evidently, all phosphoryla-tions of transcription-related proteins must be removed, at some point, by one or more phosphatases. For example, loss of PP1 results in increased phosphorylation of multiple residues of the transcription factor and proto-oncogene product MYC (Dingar et al. 2018). Nevertheless, we focus here on phosphatases that work on the core transcription machinery to regulate progression through the RNAPII cycle, as opposed to accessory factors such as activators or coactivators. We begin with the specialized or dedicated enzymes that were identified in targeted biochemical and genetic studies, and conclude with the PPP family, the transcriptional roles of which were initially obscured by their functional pleiotropy.

FCP1 and family: the original CTD phosphatases

The emergence of RPB1-CTD phosphorylation as a key regulator of transcription set off hunts for the kinases and phosphatases involved. Purification of the major CTD phosphatase detectable in whole-cell extracts led to the identification of budding yeast Fcp1. Mass spectrometric characterization of Fcp1-containing complexes revealed an interaction with the Rap74 subunit of the GTF TFIIF, which functions in both initiation and elongation; a yeast two-hybrid interaction screen with TFIIF
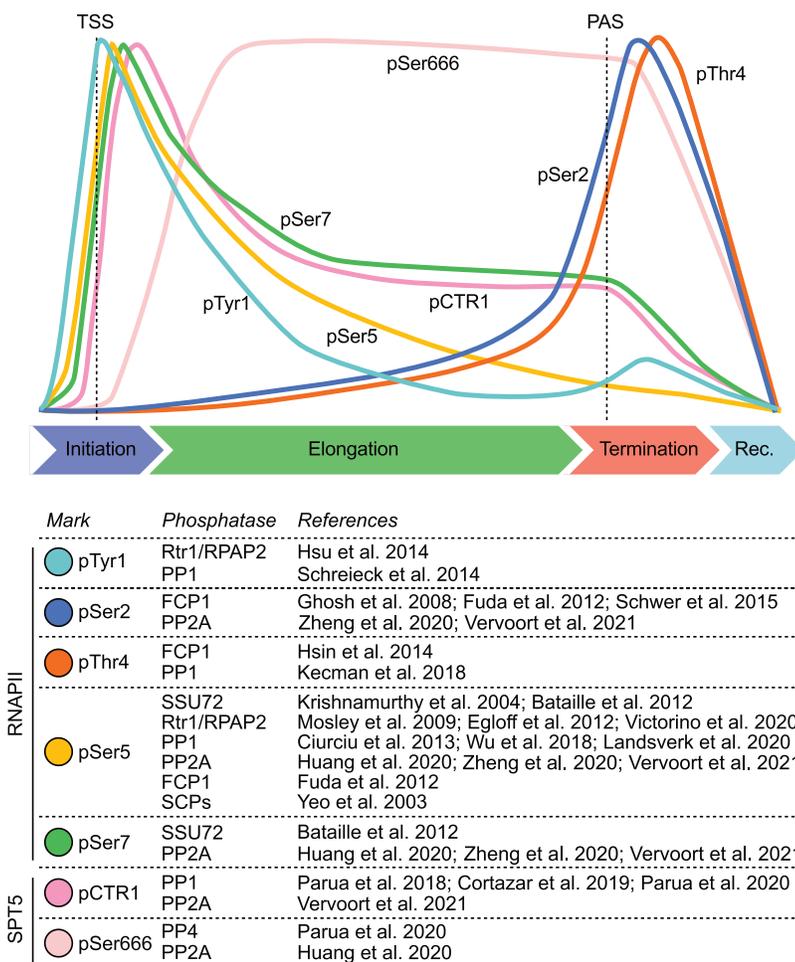

Figure 4. Phosphatases target the RNAPII CTD and SPT5 throughout the transcription cycle. (*Top*) Typical profiles of indicated RNAPII and SPT5 phosphorylation marks along the bodies of genes in higher eukaryotes. Due to the high variability of pTyr1 profiles across species, the one depicted is based on the distribution reported in metazoans (Descostes et al. 2014). (*Bottom*) For each mark, proposed phosphatases are indicated. For PPPs, only catalytic subunits are reported. (TSS) Transcription start site, (PAS) polyadenylation signal.

	Mark	Phosphatase	References
RNAPII	pTyr1	Rtr1/RPAP2 PP1	Hsu et al. 2014 Schreieck et al. 2014
	pSer2	FCP1 PP2A	Ghosh et al. 2008; Fuda et al. 2012; Schwer et al. 2015 Zheng et al. 2020; Vervoort et al. 2021
	pThr4	FCP1 PP1	Hsin et al. 2014 Kecman et al. 2018
	pSer5	SSU72 Rtr1/RPAP2 PP1 PP2A FCP1 SCPs	Krishnamurthy et al. 2004; Bataille et al. 2012 Mosley et al. 2009; Egloff et al. 2012; Victorino et al. 2020 Ciurciu et al. 2013; Wu et al. 2018; Landsverk et al. 2020 Huang et al. 2020; Zheng et al. 2020; Vervoort et al. 2021 Fuda et al. 2012 Yeo et al. 2003
	pSer7	SSU72 PP2A	Bataille et al. 2012 Huang et al. 2020; Zheng et al. 2020; Vervoort et al. 2021
SPT5	pCTR1	PP1 PP2A	Parua et al. 2018; Cortazar et al. 2019; Parua et al. 2020 Vervoort et al. 2021
	pSer666	PP4 PP2A	Parua et al. 2020 Huang et al. 2020

subsequently identified human FCP1 (Chambers and Dahmus 1994; Chambers et al. 1995; Chambers and Kane 1996; Archambault et al. 1997, 1998). Fcp1 is encoded by an essential gene in budding yeast, and thermal inactivation of a temperature-sensitive mutant variant produced decreases in mRNA levels and RNAPII occupancy on chromatin consistent with transcription failure (Kobor et al. 1999). More than two decades later, the mechanistic basis for this requirement remains only partly understood. Early studies in vitro indicated a role for human FCP1 in reinitiation by RNAPII after completing a round of transcription; in a defined system consisting of purified factors, the addition of FCP1 allowed the formation of a competent PIC when the only source of RNAPII was the hyperphosphorylated II_0 form, consistent with an ability of FCP1 to dephosphorylate the CTD of RNAPII while it is off chromatin (Cho et al. 1999). Similarly, Fcp1 depletion by RNA interference (RNAi) in *Drosophila* S2 cells selectively decreased RNAPII density and mRNA synthesis at the heavily transcribed heat shock genes; phosphorylation increased on both Ser2 and Ser5 residues, specifically in the chromatin-free fraction (Fuda et al. 2012). These and other studies suggest that an important function of FCP1 is to ensure a supply of unphosphorylated RNAPII (II_A form) to initiate at promoters of highly expressed genes.

Multiple studies suggest additional, albeit less clearly defined, roles for FCP1 during the elongation phase. In vitro, human FCP1 stimulated elongation, although this effect was independent of its phosphatase activity (Cho et al. 1999; Mandal et al. 2002). In vivo, FCP1 can be cross-linked to chromatin throughout the bodies of genes in both yeast and metazoan systems (Fuda et al. 2012; Zhang et al. 2012). In budding yeast (as in other systems), pSer5 plays a conserved, essential role in recruitment of the mRNA 5′ end capping enzymes, and Fcp1 is needed to promote CTD dephosphorylation and, possibly as a consequence, the timely release of capping enzymes from the elongating RNAPII complex (Schroeder et al. 2000). In another study in budding yeast, shifting an *fcp1-ts* mutant to restrictive temperature diminished total RNAPII occupancy but selectively increased pSer2—a mark associated with elongation—on chromatin of active genes, suggesting a continued requirement for Fcp1 activity even after initiation (Cho et al. 2001). That effect of Fcp1 inactivation on pSer2 was unchanged by mutation of *kin28* or *srb10* (encoding the CDK7 or CDK8 ortholog, respectively) but was abolished by the deletion of *ctk1* (CDK12 ortholog). Interestingly, Ctk1, like Fcp1, cross-links along the lengths of gene coding regions, suggesting that the lack of spatial correlation between Ctk1 and its product pSer2, which increases toward 3′ ends (Fig. 4), might be due to Fcp1 removing this mark as quickly as it is placed by Ctk1 in more upstream regions.

A preference for pSer2 over pSer5 as a substrate is determined by unique structural features of FCP1. Budding yeast Fcp1 contains at its C terminus a BRCA1 C-terminal (BRCT) domain, which is essential for viability and required for activity in vitro (Kobor et al. 2000). Typically, BRCT domains occur in tandem pairs that form phosphoprotein-binding motifs (Manke et al. 2003). In the X-ray crystal structure of fission yeast Fcp1, the single BRCT domain forms one wall of a deep canyon with the active site at its floor (Ghosh et al. 2008) and confers a strong, albeit not absolute, substrate preference for pSer2-containing repeats. Amino acid substitutions in Fcp1 that enhance this preference and virtually abolish the ability of the enzyme to dephosphorylate pSer5-containing substrates had no obvious phenotypic effects in *S. cerevisiae* (Ghosh et al. 2008) but were lethal in *S. pombe* and suppressed by mutations that shorten the Rpb1-CTD (Schwer et al. 2015). Neither an *rpb1-S2A* mutation that replaces all Ser2 positions with Ala (and is viable in fission yeast) nor an *rpb1-S5A-MCE* mutation in which the lethality of the Ser5 → Ala substitution is suppressed by fusion of Rpb1 to mammalian capping enzyme obviated the essential requirement for Fcp1 function. Therefore, lethality due to loss of Fcp1 in fission yeast cannot be ascribed solely to dysregulation of either pSer2 or pSer5. FCP1 has also been implicated in dephosphorylation of pThr4 of the CTD in human cells (Hsin et al. 2014) and might have transcriptionally relevant substrates apart from RNAPII, possibly including Spt5 (Schwer et al. 2015). Therefore, although FCP1 clearly has a conserved function in removing pSer2 and facilitating recycling of RNAPII after rounds of productive elongation, there is abundant evidence—both genetic and biochemical—hinting at a more expansive portfolio for this phosphatase during the transcription cycle.

The SCPs (encoded in human by the genes *CTDSP1*, *CTDSP2*, *CTDSPL*, and *CTDSPL2*) were identified on the basis of homology with FCP1 and shown to have CTD phosphatase activity in vitro, but with a preference for pSer5 over pSer2 (Yeo et al. 2003). Human SCP1/CTDSP1 is similar in structure to FCP1 but lacks a BRCT domain, allowing a different mode of phospho-CTD substrate binding and likely accounting for the different specificities of the two enzymes for pSer2 versus pSer5 (Kamenski et al. 2004; Ghosh et al. 2008). SCPs do not appear to play a major role in transcription, as suggested by early loss-of-function studies (Yeo et al. 2005). Interestingly, SCP expression is restricted to nonneuronal lineages, where they promote the silencing of a specific subset of genes involved in neuronal development, presumably by locally maintaining RNAPII in unphosphorylated form (Yeo et al. 2005; Xue et al. 2013; Nesti et al. 2014).

Rtr1 and RPAP2: founding members of a new phosphatase family

Wild-type FCP1 has only weak activity toward pSer5 in vitro, and in most studies, inactivation or depletion of FCP1 preferentially affected pSer2 (and pThr4) levels in vivo, leaving open a role for a pSer5 phosphatase. Such an activity might be needed to (1) remove phosphates from the CTD of RNAPII after escape from the promoter region, where pSer5 generally peaks (Fig. 4; Kim et al. 2010; Mayer et al. 2010; Tietjen et al. 2010; Bataille et al. 2012), or (2) promote reinitiation by dephosphorylating pSer5 of soluble RNAPII, possibly in conjunction with a pSer2-specific

enzyme such as FCP1 (see above). One candidate emerged with the identification and characterization of Rtr1, the budding yeast ortholog of the human RNAPII-associated protein 2 (RPAP2), which had been detected, but not functionally characterized, in an earlier proteomic analysis (Jeronimo et al. 2007). The *RTR1* gene of *S. cerevisiae* was implicated in transcription and stress tolerance and shown to interact genetically with components of the RNAPII machinery including Mediator, Spt4/Spt5, and the PAF complex (Gibney et al. 2008). Its protein product bound both RNAPII and pSer5-containing phosphopeptides, and cross-linked to chromatin of active genes between the peaks of pSer5 and pSer2 (Fig. 4), suggesting a role in cotranscriptional pSer5 removal (Mosley et al. 2009). In *rtr1* deletion strains, pSer5 levels increased both in extracts and on chromatin, and there was evidence of transcriptional impairment—reductions in specific mRNAs coinciding with decreased occupancy by RNAPII, and readthrough transcription of certain genes suggestive of a termination defect. This study detected a pSer5-specific phosphatase activity associated with Rtr1 purified from yeast extracts, but a structural and biochemical analysis of the *Kluyveromyces lactis* enzyme raised doubts about whether it possessed an identifiable active site or intrinsic phosphatase activity (Xiang et al. 2012); another study suggested that *K. lactis* Rtr1 might be especially prone to inactivation during purification (Hsu et al. 2014). A structure of the *S. cerevisiae* Rtr1 resolved this uncertainty by revealing a novel active site for phosphoryl transfer, definitively demonstrating intrinsic activity in vitro and firmly establishing Rtr1 and RPAP2 as founding members of yet another class of non-PPPs involved in transcription (Fig. 2; Irani et al. 2016).

In vitro, activity of the purified Rtr1 monomer toward model substrates was weak (Irani et al. 2016), suggesting that additional protein–protein interactions or accessory subunits might facilitate its function in vivo. Additional complexity was also suggested by studies implicating RPAP2 in 3′ end processing of snRNA genes via its association with the Integrator complex; RPAP2 specifically binds pSer7, previously known to promote Integrator recruitment, and depletion of RPAP2 mimicked effects of Ser7 → Ala substitutions in the CTD by increasing levels of pSer5, both in extracts and on chromatin at the gene encoding U2 snRNA (Egloff et al. 2012). Importantly, pSer7 was not required for RPAP2 occupancy of the *ACTB* gene (where pSer5 also increased upon RPAP2 knockdown), indicating that there are multiple mechanisms for recruitment of the phosphatase to RNAPII transcribed genes. Beyond pSer5, pTyr1 might be a target of Rtr1 (Hsu et al. 2014). Moreover, two CTD-interacting domain (CID)-containing proteins, RPRD1A and RPRD1B, were shown to form both heterodimers and homodimers that bind preferentially to pSer2- or pSer7-containing repeats in vitro, erecting a "scaffold" to recruit RPAP2 and thereby stimulate it to dephosphorylate pSer5 on an adjacent repeat; depletion by RNAi indicated that the RPRDs also mediate RPAP2 interaction with RNAPII in vivo (Ni et al. 2014).

Even harder to pin down than their enzymatic activity have been the precise functions of Rtr1 and RPAP2 in

gene expression and cellular physiology. A recent study combining proteomics, genomics, and bioinformatics suggested roles of Rtr1 and pSer5 in determining termination pathway choice at genes transcribed by RNAPII in budding yeast (Victorino et al. 2020). In *rtr1* deletion strains with elevated levels of pSer5, interactions of the CTD with cleavage factor Ia (CFIa) were diminished, while those with another termination factor—Nrd1, which preferentially binds pSer5-containing repeats—were increased. Nrd1 is required, together with Nab3, Sen1, and additional factors, in the NNS pathway, an alternative termination mechanism in yeast. Loss of Rtr1 altered the distribution of RNAPII on protein-coding genes, shifting it toward the TSS, consistent with premature termination, possibly via engagement of NNS factors. Interestingly, loss of Ssu72, another pSer5-specific phosphatase residing in the cleavage and polyadenylation factor (CPF) required for termination of polyadenylated transcripts (discussed in the next section), has roughly the opposite effect: impaired, rather than premature, termination. A clear message that emerges from this cloudy picture is that the function of transcription-associated phosphatases is not dictated solely by their specificities for positions within the CTD repeat.

SSU72 links the beginning and end of transcription

Ssu72 is a pSer5-specific phosphatase, which was affinity-purified as a stoichiometric subunit of the CPF in budding yeast (Dichtl et al. 2002) and fission yeast (Vanoosthuyse et al. 2014). Interestingly, the *SSU72* gene was first characterized on the basis of a genetic interaction with a GTF required for initiation, TFIIB, encoded by the *SUA7* gene in budding yeast; an *ssu72* mutation enhanced a defect in TSS specification of a *sua7* mutant, presaging a linkage between the initiation and termination steps of transcription (Sun and Hampsey 1996). Ssu72 is essential for viability in *S. cerevisiae* (but not in *S. pombe*), and temperature-sensitive or inducibly degraded mutant variants elicit a constellation of transcription-associated phenotypes: extensive readthrough transcription indicative of a general termination defect (Dichtl et al. 2002), a specific defect in snRNA termination similar to that seen in NNS pathway mutants (Ganem et al. 2003), increased pSer5 (but not pSer2) (Krishnamurthy et al. 2004; Reyes-Reyes and Hampsey 2007), and a loss of the transcription-dependent physical interaction between promoter and terminator regions, or "gene looping," detected by chromosome conformation capture (3C) analysis (Ansari and Hampsey 2005; Tan-Wong et al. 2012).

Ssu72 can also remove pSer7, another phosphate mark on the RNAPII CTD that is placed by both Cdk7 and Cdk9 orthologs (Glover-Cutter et al. 2009; St. Amour et al. 2012; Zhang et al. 2012). In budding yeast, both pSer5 and pSer7 decrease ~350 bp upstream of the polyadenylation signal (PAS) (Fig. 4) but persist beyond the PAS in *ssu72* mutants (Bataille et al. 2012). Failure of pSer7 dephosphorylation in *ssu72* mutants was suggested to contribute to their associated defects in transcription termination (Zhang et al. 2012).

Ssu72 contains a CX$_5$R motif typically found in protein tyrosine phosphatases (PTPases); mutation of the catalytic Cys residue abolished activity toward *p*-nitrophenylphosphate and CTD-derived substrates in vitro (Meinhart et al. 2003; Krishnamurthy et al. 2004). A Cys → Ser substitution at this position was lethal in *S. cerevisiae* (Sun and Hampsey 1996). A catalytically dead C15S variant also failed to restore normal levels of pSer5 after depletion of an Ssu72-degron fusion protein but was able to rescue 3′ end processing of transcripts in an Ssu72-depleted extract, possibly reflecting a structural role of the protein in maintaining CPF integrity. In contrast, Ssu72 depletion impaired promoter-dependent transcription in yeast extracts, with a concomitant increase in pSer5 and depletion of RNAPII$_A$, and these defects were rescued by wild-type but not catalytically inactive Ssu72 (Krishnamurthy et al. 2004). Similarly, gene looping depends on Ssu72 catalytic activity and on the core CPF subunit Pta1, both of which associate with promoter and terminator regions but not with intervening gene bodies (Ansari and Hampsey 2005). In yeast, gene looping—and the *cis* and *trans* regulators that enforce it, which include the promoter, PAS, TFIIB, and CPF—is proposed to enforce directionality of transcription from intrinsically bidirectional promoters and thereby prevent transcriptional interference in tandem gene pairs (Tan-Wong et al. 2012). More generally, juxtaposing terminators and promoters would be an efficient means to recycle RNAPII for new rounds of transcription—a role that might require CTD phosphatases such as Ssu72 to erase phosphate marks placed in the previous cycle. Gene looping per se has not been observed in metazoans, but it seems likely that phosphatases such as SSU72 will play prominent roles in RNAPII recycling mechanisms yet to be uncovered.

PPPs emerge as key regulators of RNAPII transcription

Ssu72 is only one of the two phosphatases resident in the CPF; both budding and fission yeast CPF complexes also contain a PP1 holoenzyme (Dichtl et al. 2002; Vanoosthuyse et al. 2014). This was the first demonstration of a PPP family member associating with the core transcriptional machinery and was a harbinger of the central roles of these enzymes now emerging throughout the RNAPII cycle. The budding yeast genome encodes a single, essential PP1 catalytic subunit, Glc7, which, together with the regulatory subunit Ref2 and other factors, forms a subcomplex within the CPF (Fig. 2; Nedea et al. 2003). Glc7 has been implicated in transcription termination, and putative targets of the phosphatase include Sen1 in the NNS termination pathway (Nedea et al. 2008) and pTyr1 of the Rpb1-CTD, removal of which is important for recruitment of the cleavage factors Rtt103 and Pcf11 (Schreieck et al. 2014). Depletion of the PP1 nuclear targeting subunit (PNUTS) led to RNAPII readthrough of termination signals in mouse cells, also consistent with a role for a PP1 holoenzyme in 3′ end formation (Austenaa et al. 2015). *S. pombe* has two genes encoding PP1 catalytic iso-

forms, *dis2*$^+$ and *sds21*$^+$, neither of which is essential for viability (Fig. 2; Ohkura et al. 1989). Only Dis2, however, is found, together with its regulatory subunits Ppn1 (ortholog of PNUTS) and Swd2.2, in the CPF, where it plays nonredundant roles in chromosome condensation and transcription termination (Vanoosthuyse et al. 2014; Parua et al. 2018). Inactivation of Dis2 in a cold-sensitive lethal *dis2-11* mutant did not affect levels of Rpb1-pTyr1 on chromatin but increased levels of phosphorylation on the C-terminal repeats (CTRs) of Spt5 and led to readthrough transcription extending beyond normal termination zones, genome-wide, detected by precision run-on transcription and sequencing (PRO-seq) (Parua et al. 2018). The Spt5 CTRs are phosphorylated by Cdk9 to accelerate elongation by RNAPII (Booth et al. 2018); their PP1-dependent dephosphorylation downstream from the PAS (Fig. 4) appears to apply a brake, leading to RNAPII accumulation in a 3′ paused complex prone to termination—a mechanism conserved between *S. pombe* (Kecman et al. 2018; Parua et al. 2018) and human cells (Fig. 5; Cortazar et al. 2019; Eaton et al. 2020; Parua et al. 2020).

PP1 regulates the RNAPII transcription cycle at multiple steps

Whereas the defined functions of yeast PP1 are executed near the end of transcription, critical roles have been ascribed to metazoan PP1 throughout the cycle. All of these functions involve the same regulatory subunit, PNUTS, encoded by *PPP1R10* (Fig. 5). A role of PP1-PNUTS during early elongation was first suggested by the apparent influence of mouse PNUTS on RNAPII transcriptional polarity; the depletion of PNUTS and of an interactor, Wdr82, induced antisense transcription from bidirectional promoters (Austenaa et al. 2015). This study did not identify the relevant PP1-PNUTS substrate, but antisense transcription induction might involve the known target Spt5, which in its unphosphorylated form restrains movement of RNAPII both upstream of and downstream from the TSS on human genes (Brannan et al. 2012) and has been implicated in suppression of antisense transcription initiating at intragenic sites in *S. pombe* (Shetty et al. 2017; Sansó et al. 2020). A role for PP1 in the promoter-proximal region is also consistent with recent ChIP-seq analysis of PNUTS, which revealed a distribution similar to that of RNAPII between the TSS and pause site (Cossa et al. 2020).

In ChIP analysis, PNUTS cross-links between the RNAPII pause site and the first exon–intron boundary. PNUTS becomes phosphorylated dependent on the kinase NUAK1 and thus more prone to bind and recruit PP1 catalytic subunits. The incoming PP1 is mainly complexed to the nuclear inhibitor of PP1 (NIPP1), a PP1 regulatory subunit involved in splicing (Fig. 5). Therefore, a NIPP1-PNUTS switch might couple splicing to RNAPII pause release in a temporally and spatially coordinated manner (Cossa et al. 2020). The NUAK1-PP1 interaction also affects later events in transcription; NUAK1 inhibition led to a loss of PNUTS binding to chromatin at both upstream sites and the 3′ ends of genes, where
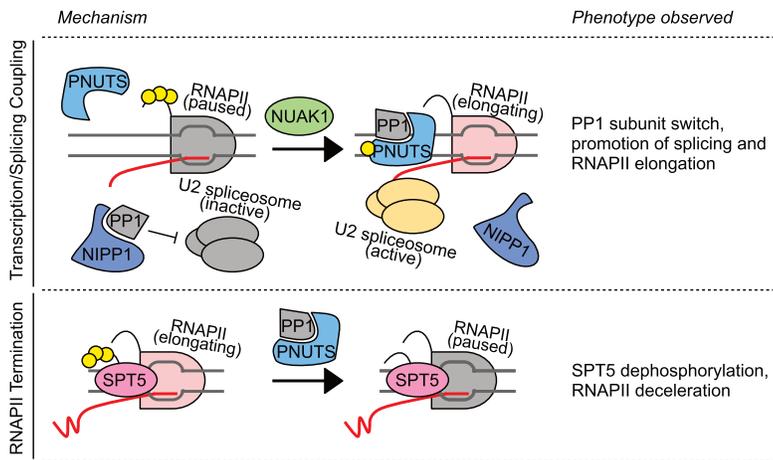
Figure 5. The PP1-PNUTS holoenzyme acts at multiple steps of transcription. (*Top*) PP1-PNUTS activity couples transcription to splicing. PNUTS is recruited to RNAPII paused at the first exon–intron boundary but lacks the PP1 catalytic subunit. Concomitantly, PP1 joins another holoenzyme (likely with the NIPP1 splicing factor) and inhibits the U2 spliceosome. The NUAK1 kinase phosphorylates PNUTS and triggers a PP1 subunit switch to bind PNUTS, thereby promoting both pause release and splicing activation (Cossa et al. 2020). (*Bottom*) PP1-PNUTS is required for efficient termination. PP1-PNUTS dephosphorylates SPT5, slowing RNAPII elongation and ensuring proper, spatially precise termination (Kecman et al. 2018; Parua et al. 2018; Cortazar et al. 2019; Cossa et al. 2020; Eaton et al. 2020; Parua et al. 2020). (Yellow circles) Phosphorylation.

nascent RNA-seq analysis indicated transcriptional read-through, in keeping with results of PNUTS depletion in mice and with a role of PP1 in termination (Fig. 5; Austenaa et al. 2015; Cortazar et al. 2019; Eaton et al. 2020; Parua et al. 2020).

Other reports have suggested a role of PP1-PNUTS as a CTD phosphatase. Embryos lacking *Drosophila* PNUTS (dPNUTS), which colocalizes and interacts with RNAPII, had increased pSer5 and impaired gene transcription (Ciurciu et al. 2013). In two recent studies, PNUTS pulldown from human cell extracts coprecipitated RNAPII, and phosphatase assays indicated selectivity for pSer5 in vitro (Wu et al. 2018; Landsverk et al. 2020). The fission yeast Dis2-Ppn1 holoenzyme has also been suggested to dephosphorylate the Rpb1-CTD, albeit on a different residue, Thr4 (Fig. 4; Kecman et al. 2018). It is unclear in either organism whether these phosphorylations increased as direct or indirect results of PP1 inactivation in vivo, and thus, the status and specificity of PP1-PNUTS as an RNAPII-CTD phosphatase remain uncertain.

RNAPII pausing and elongation control: a job opening for PPP family phosphatases

After initiation and promoter clearance on a large fraction of human genes, RNAPII transcribes ~50–100 nt and becomes stably paused, awaiting additional signals to trigger resumption of processive elongation. This promoter-proximal pausing underlies a distinctive feature of RNAPII ChIP profiles in metazoans: a sharp peak in occupancy just downstream from the TSS on pause-regulated genes. Different organisms and cell types have different proportions of paused versus nonpaused genes (at which RNAPII recruitment or initiation is limiting for expression), typically differentiated by the ratio of RNAPII in the TSS region versus the gene body, known as a pause index (Core and Adelman 2019). Promoter-proximal pausing is nonetheless a pervasive feature of gene regulation in metazoans and also occurs in fission yeast (but not budding yeast), albeit at a smaller fraction of genes (Booth et al. 2016). Importantly, the machinery that governs

pausing—pause-inducing and -releasing factors, and the kinases and phosphatases that modify them—is conserved in all eukaryotes, including fungi that lack detectable pausing (with the exception of the negative elongation factor NELF, which is absent in yeast, plants, and worms). Moreover, that machinery appears to be engaged—and Cdk9 activity is required for normal elongation—at virtually all genes transcribed by RNAPII, whether or not release from the pause is the rate-limiting step in their expression (Jonkers et al. 2014; Booth et al. 2018; Luse et al. 2020). The significance of pausing in regulating gene expression has been inferred mainly from the classes of genes most likely to harbor a paused RNAPII elongation complex; highly paused gene subsets are typically enriched for ones involved in stimulus responses or cell cycle regulation, possibly because the release of paused RNAPII, rather than recruitment and initiation de novo, allows a faster or more switch-like ("all or none") transcriptional response (Core and Adelman 2019).

The mechanisms underlying pausing and pause release have been extensively studied. Both steps—establishment and release—are phosphorylation-dependent and thus regulated by kinases and phosphatases in vivo. A paused RNAPII ternary complex was reconstituted in vitro by addition of DSIF and NELF (Vos et al. 2018b). This complex was severely impaired for transcript elongation, relative to the one containing RNAPII alone, but could be activated by the addition of P-TEFb together with the elongation-promoting factors SPT6 and the PAF complex. This activation, which is likely to mimic pause release in vivo, was accompanied by CDK9-mediated phosphorylation of RNAPII (on both the CTD and a linker region connecting the CTD to the rest of RPB1), DSIF, NELF, SPT6, and PAF complex subunits. Phosphorylated DSIF, SPT6, and PAF were all retained in the active elongation complex, whereas NELF was released (Vos et al. 2018a).

After pause release and during elongation in vivo, Ser5 becomes progressively dephosphorylated (Fig. 4), making pSer5 phosphatases candidates for a role in pause release. No added phosphatase was needed, however, to convert a

paused RNAPII elongation complex to an actively elongating one in a defined system comprising purified components (Vos et al. 2018a). Moreover, in ChIP analysis, pSer5 undergoes a gradual decrease rather than a sharp drop in occupancy downstream from the pause site, suggesting that pSer5 removal is not a strict prerequisite for efficient elongation (Bataille et al. 2012). To date, studies of the dedicated pSer5 phosphatases SSU72, Rtr1/RPAP2, and the SCPs have left uncertain their functional relevance to RNAPII pausing and elongation control. It is now clear, however, that other phosphatases—and other targets— play decisive roles in these events. In particular, PPPs have emerged as the major phosphatases that oppose kinase-driven elongation by RNAPII. These studies specifically implicate PP1, PP4, and PP2A, acting through the CTD, SPT5, and possibly other targets, as gatekeepers of RNAPII pause release and brakes on RNAPII elongation (Fig. 4).

PP4 and PP2A keep RNAPII in the starting blocks

Beyond PP1, two other PPP family phosphatases have recently emerged as regulators of early steps in the RNAPII transcription cycle: PP4 and PP2A. By orthogonal approaches in different organisms, two groups independently identified PP4 as a phospho-SPT5 phosphatase. In the worm *Caenorhabditis elegans*, expression of certain stress-resistance and longevity-promoting genes driven by the transcription factor DAF-16 is dependent on SMK-1, which is a regulatory subunit of a PP4 holoenzyme (ortholog of the human *PPP4R3B* gene product). Depletion of PP4 subunits by RNAi led to increased phosphorylation on multiple sites in SPT-5 and impaired transcription of specific DAF-16 target genes, although the block appeared to be at initiation rather than during elongation (Sen et al. 2020). Independently, a human PP4 complex was shown to be a target of negative regulation by CDK9, through an inhibitory phosphorylation on another regulatory subunit, PPP4R2, first detected in a chemical-genetic screen for CDK9 substrates (Sansó et al. 2016). Both PPP4R2 and -3 were previously shown to be phosphorylated by a cell cycle CDK to inhibit PP4 and regulate microtubule nucleation at centrosomes (Voss et al. 2013). Treatment of human cells with a CDK9 inhibitor diminished PPP4R2-Thr173 phosphorylation on chromatin, and increased phosphatase activity recovered in anti-PP4 immunoprecipitates, relative to mock-treated controls. Conversely, treatment of PP4 complexes with purified P-TEFb inhibited their dephosphorylating activity toward phosphopeptide substrates. In vitro, PP4 dephosphorylated a site on SPT5 that was refractory to PP1 (Parua et al. 2020). Both that site—pSer666 (Fig. 4), within a linker connecting conserved KOW4 and KOW5 motifs—and sites within CTR1 were phosphorylated when paused complexes were converted to actively elongating ones by P-TEFb in vitro (Figs. 4, 6; Vos et al. 2018a). PP4 complex subunits cross-linked to the promoter-proximal regions of human genes, and their depletion by RNAi increased SPT5 phosphorylation on Ser666 (and CTR1) and caused changes in RNAPII distribution, suggesting attenuation

of promoter-proximal pausing. This pattern, detected on three highly paused genes, suggests that human PP4 is a negative regulator of RNAPII entry into processive elongation and a potential enforcer of promoter-proximal pausing (Parua et al. 2020). Taken together, the results in worms and humans indicate a conserved function of a PP4 holoenzyme in dephosphorylating SPT5 and possibly other components of the paused complex to regulate early steps in RNAPII transcription.

Now, three independent and mutually reinforcing studies have implicated PP2A in key aspects of RNAPII transcription, including pause regulation, elongation, and termination (Huang et al. 2020; Zheng et al. 2020; Vervoort et al. 2021). One analysis, in *Drosophila* cells in culture, uncovered a large number of protein-coding genes that were derepressed by depletion of INTS8, a subunit of the Integrator complex (Huang et al. 2020). Proteomic analysis revealed an interaction of INTS8 with both the A (PR65) and C subunits of PP2A (as well as other Integrator subunits) but no identifiable, regulatory B subunit. Instead,
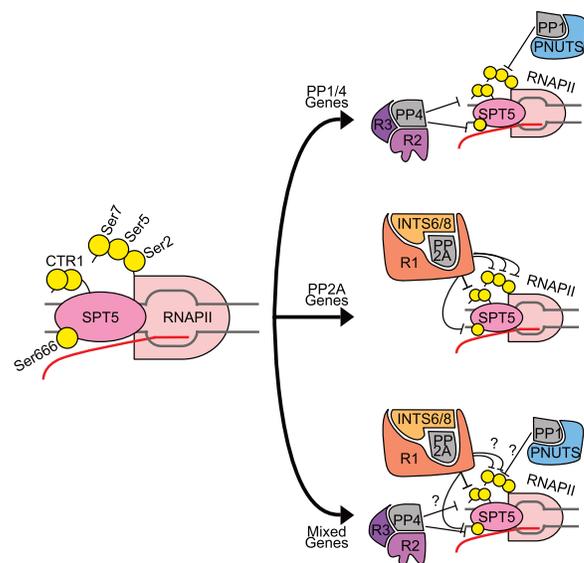

Figure 6. Different PPP holoenzymes cooperate during RNAPII pausing. (*Left*) Relevant phosphorylation sites on SPT5 and RNAPII. SPT5 C-terminal repeat region 1 (CTR1) typically contains Thr-Pro repeats, where Thr can be phosphorylated by CDK9 (e.g., Thr806 in human SPT5). SPT5 contains another region— the KOW4–KOW5 linker—with multiple Ser residues phosphorylated by CDK9 (including Ser666 in human SPT5). The RNAPII CTD contains multiple heptad repeats, which include phosphorylated residues Ser2, Ser5, and Ser7 (Tyr1 and Thr4 are omitted for clarity). Different PPP family phosphatases have been proposed to act at the promoter-proximal pause, as indicated at *right*. We speculate that these enzymes might act at different, hypothetical gene classes: "PP1/PP4 genes," at which PP4 targets SPT5 (Parua et al. 2020) and PP1-PNUTS dephosphorylates pSer5 (Wu et al. 2018; Landsverk et al. 2020); "PP2A genes," at which PP2A-PPP2R1A, associated with the Integrator, targets both SPT5 and RNAPII residues (Huang et al. 2020; Zheng et al. 2020; Vervoort et al. 2021), and "mixed genes," where RNAPII and SPT5 are targeted by PP1, PP2A, and PP4 holoenzymes.

INTS8 appears to provide surrogate B subunit function within the Integrator–PP2A complex; mutating four residues in a short PP2A-recruiting motif of INTS8 (WFEF) to Ala led to up-regulation of Integrator target genes, mimicking knockdown of INTS8, PP2A-C, or PP2A-A or inhibition of PP2A with small molecules. The mechanism of increased transcription appeared to be enhanced release from the promoter-proximal pause; of the most highly paused genes (top decile of genes ranked by pause index), over half were induced by shRNA targeting INTS8, and PRO-seq analysis revealed redistribution of RNAPII away from the pause site when PP2A recruitment was prevented. Similar results were obtained in human embryonic kidney (HEK) 293T cells. Interestingly, perturbation of Integrator–PP2A activity or integrity in cells caused selective increases in SPT5 phosphorylation on Ser666 (or its equivalent residue in *Drosophila* SPT5), but not on C-terminal repeat region 1 (CTR1), and on both Ser5 and Ser7 (but not Ser2) of the RNAPII CTD (Fig. 4; Huang et al. 2020).

In a parallel study, a proteomic search for binding partners of nuclear PP2A in human cells yielded subunits of the Integrator (Zheng et al. 2020). In agreement with the previous work, this Integrator–PP2A complex (termed "INTAC"), recovered from human cell extracts, contained A and C but not B subunits. Moreover, in a cryo-electron microscopy (cryo-EM) structure of Integrator bound to PP2A-A and -C subunits, the possibility of a "missing" B subunit was effectively excluded due to steric constraints. Instead, the canonical functions of the B subunit in substrate selection are likely to be performed by the Integrator subunits that directly contact PP2A: INTS8, which provides a PP2A-docking site on Integrator, and INTS6, which is even more intimately associated with the PP2A core in a phosphatase module that occupies one side of a cruciform structure comprising backbone and (INTS8-containing) shoulder subcomplexes. The endonuclease subunit INTS11, implicated in premature termination of promoter-proximally engaged RNAPII and in snRNA 3′ end formation (Elrod et al. 2019; Tatomer et al. 2019), binds to the other side of the cruciform. In ChIP-seq analysis, PP2A recruitment to chromatin was dependent on the Integrator subunits but not vice versa. Specific loss of PP2A recruitment led to up-regulation of Integrator target genes, but a preferential effect on pausing or elongation was not detected in this study. In vitro, the purified INTAC holoenzyme dephosphorylated pSer2, pSer5, and pSer7 of RNAPII phosphorylated by TFIIH, whereas in vivo, depletion of PP2A or INTS8 subunits increased pSer2 and pSer5 ChIP signals at target genes. SPT5 was not evaluated as a PP2A target in this study, either in vivo or in vitro.

A third study reinforces the central observation of an Integrator–PP2A holoenzyme with key roles in the RNAPII transcription cycle while providing strong evidence that this complex works in specific opposition to P-TEFb (Vervoort et al. 2021). Approaching the question from yet a third angle, the investigators performed screens of CRISPR-mediated gene knockouts in leukemia-derived cell lines to identify mechanisms of resistance to CDK9 inhibitors. Among the top hits were both INTS6 and INTS8. After identifying, validating, and characterizing an Inte-

grator–PP2A complex in human-cell extracts (also lacking any evidence of a B subunit), the investigators implicated this complex in regulation of RNAPII transcription genome-wide. In ChIP-seq analysis, RNAPII, Integrator, and PP2A colocalized at all active genes, and PP2A was recruited de novo to stimulus-responsive genes concomitant with their transcriptional activation by lipopolysaccharide or epidermal growth factor treatments. These results suggested a specific role of PP2A in antagonizing CDK9; impairing PP2A function, through knockout or knockdown of INTS6 or INTS8 or through pharmacological phosphatase inhibition, overcame effects of CDK9 inhibitors on target protein phosphorylation—including Ser2, Ser5, and Ser7 of the CTD and CTR1 of SPT5—and on transcription by RNAPII. Preventing PP2A recruitment rescued the decreased levels of nascent transcription and attenuated the enhancement of promoter-proximal pausing elicited by CDK9 inhibition, likely explaining the CDK9 inhibitor resistance conferred by INTS6 or INTS8 knockouts. Conversely, treating cells with a small-molecule activator of PP2A (SMAP) further enhanced pausing due to CDK9 inhibition and synergized with CDK9 inhibitors in killing human tumor cells both in culture and in mouse xenograft models.

The pause-enforcing functions and phosphoprotein substrates assigned to the Integrator–PP2A complex overlap with those inferred for PP4 complexes in human cells (Parua et al. 2020). The simplest way to rationalize the existence of two pause-enhancing phosphatases is that each acts at a distinct subset of genes, possibly to promote transcriptional responses to different environmental stimuli (Fig. 6, top and middle). Different analyses have led to subtly different conclusions about the ubiquity of PP2A on protein-coding genes, and genome-wide analyses of PP4 occupancy have yet to be reported. Another, more interesting (and maybe more likely) possibility is that PP2A and PP4 might cohabitate on many or even most genes, either to respond to different stimuli or to work together (Fig. 6, bottom). In this light, it is intriguing that immunoprecipitates of SMK-1 from *C. elegans* extracts contained, in addition to PP4 subunits, the PP2A scaffold subunit PAA-1 (Sen et al. 2020). Future investigations should aim to reveal the division of labor among different PPP family members in restraining elongation at the promoter-proximal pause and, possibly, other transitions in the RNAPII cycle.

Taken together, these recent studies implicate both PP4 and PP2A in promoter-proximal pausing, but it has yet to be determined whether dephosphorylating enzymes are needed in vivo to establish or maintain the stably paused complex (or both). Whereas the P-TEFb-dependent activation of RNAPII elongation complexes reconstituted in vitro is likely to resemble physiologic pause release, the paused complex on which CDK9 acted was assembled with the constituent proteins purified in their unphosphorylated form (Vos et al. 2018a,b). It seems highly likely that phosphatases of the PPP family will prove to be instrumental, either individually or in combination, in opposing kinase functions in both promoter-proximal pausing and pause release in vivo, but definitive tests of

these requirements might only come from more complex systems that can recapitulate phosphoregulation of both steps in vitro.

Next steps: challenges met and unmet in the study of PPPs

The unique strategies by which PPPs achieve functional specificity and their ubiquitous roles in cell physiology render them particularly challenging to study, in contrast to non-PPPs such as FCP1, Rtr1/RPAP2, and SSU72, with more restricted or even dedicated roles in the RNAPII transcription cycle. These phosphatases are not as abundant as PP1 or PP2A (Fig. 2), but their manipulation led to phenotypes more readily interpreted as owing to transcriptional perturbation.

A more surgical approach to dissection of PPP function might be achieved through targeting individual regulatory subunits, but here too, there are challenges. First, the sheer number of PIPs that associate with PP1 appears to be huge and may be underestimated; lack of sequence conservation makes their identification difficult (Verbinnen et al. 2017). Contrast this situation with that of kinases that depend on binding to a regulatory subunit for biological activity: a typical CDK partners with only one or a few cyclins, which in most cases are readily identifiable as such by structural homology. In addition, the depletion of one phosphatase regulatory subunit affects the balance of others, such that the liberated catalytic subunit is redistributed among other partners, potentially influencing unrelated cellular events. One recent advance on this front is the development of expression systems comprising a catalytic subunit covalently linked to a regulatory one (Wu et al. 2018). This approach allows the study of individual regulatory subunits without unbalancing endogenous holoenzymes and was recently used to address the role of human PP1-PNUTS in termination (Cortazar et al. 2019).

Another key difference between kinases and phosphatases is in their "druggability"; i.e., their susceptibility to selective inhibition by small molecules. Compounds such as okadaic acid and calyculin A have been used to dissect the functions of different PPPs, but their specificity is limited: Whereas both compounds preferentially target PP2A over PP1, they are likely to inhibit both enzymes at the concentrations typically used in cell-based experiments. Moreover, active site-directed compounds cannot distinguish among different holoenzymes containing the same catalytic subunit. Attempts to use PPP inhibitors therapeutically are also problematic, as targeting of the PP1 or PP2A catalytic center can have pleiotropic, potentially toxic effects. A more promising approach, with molecules targeting specific holoenzymes, has been pioneered by several laboratories in recent years and is explored below.

Can phosphatases in transcription be targeted therapeutically?

The RNAPII machinery is an attractive target for cancer therapeutics, because cancer cells can become dependent on high-level transcription driven, for example, by oncogenic transcription factors (Bradner et al. 2017). That CDKs acting in the transcription cycle might be targeted therapeutically was first indicated by the cancer-specific cytotoxic effects of the covalent kinase inhibitor THZ1, which is specific for CDK7, CDK12, and CDK13 (Chipumuro et al. 2014; Christensen et al. 2014; Kwiatkowski et al. 2014; Wang et al. 2015). Small molecules more selective for CDK7 were less effective on their own but could be combined with compounds targeting other CDKs, or other pathways, to achieve synergistic effects, suggesting that multiple enzymatic activities might need to be ablated for the best therapeutic effects (Kalan et al. 2017; Minzel et al. 2018; Olson et al. 2019). Similarly, new insights into transcriptional phosphatase function might be harnessed to target cancer, for example, with inhibitors of NUAK1, the kinase that regulates activity of PP1 in splicing (Cossa et al. 2020).

Although compounds directly targeting the catalytic centers of PPPs are likely to have wide-ranging effects in cells and toxicity in clinical settings, alternative strategies have recently been developed to target PP2A, and promising approaches have been proposed for PP1 inhibition. PP2A dysregulation in multiple diseases is well established. For example, levels of PP2A are commonly decreased in cancer or neurodegenerative diseases, and compounds have been developed that boost its activity (Sangodkar et al. 2016). PP2A is inactivated by interacting proteins such as SET and CIP2A. Several compounds have been identified on the basis of their ability to disrupt SET/PP2A or CIP2A/PP2A complexes. This disruption is typically an indirect effect of molecules targeting other activities; for example, the proteasome inhibitor bortezomib or the EGFR inhibitor erlotinib, both of which induce CIP2A down-regulation. An additional approach is the targeting of PME1, an esterase that counteracts a carboxymethylation reaction required for PP2A activation (O'Connor et al. 2018).

An exciting recent advance was the development of SMAPs (see above)—derivatives of phenothiazines (PPZs), compounds historically used for neurological or psychiatric illnesses, which displayed the off-target effect of activating PP2A. PPZs were therefore optimized to remove their psychotropic activity and increase selectivity for PP2A (Sangodkar et al. 2017). A recent report investigated the mechanism of SMAP action, taking advantage of a cryo-EM structure of a PP2A trimer containing the prototypical SMAP DT-061 (Leonard et al. 2020). Interestingly, DT-061 bound a pocket composed of residues in all three subunits, suggesting that SMAPs can act as "molecular glue," promoting the allosteric activation of PP2A holoenzymes (Leonard et al. 2020; Westermarck and Neel 2020). A concurrent report independently described iHAP1, a SMAP mechanistically distinct from DT-061 with promising efficacy in T-cell lymphoblastic leukemia models (Morita et al. 2020). These studies show the potential of SMAPs for therapeutic PP2A reactivation. Indeed, as described above, the reactivation of Integrator–PP2A complexes with a SMAP was recently shown to synergize with CDK9

inhibitors to trigger apoptosis in cancer cells (Vervoort et al. 2021).

The development of drugs targeting PP1 holoenzymes lagged behind similar efforts aimed at PP2A. Attempts to target PP1 catalytic subunits with derivatives of natural compounds such as calyculin A were largely unsuccessful, indicating that selective PP1 inhibition might not be feasible (Vagnarelli and Alessi 2018). PP1 holoenzymes are structurally and functionally more diverse than PP2A complexes, which have a more constant quaternary structure (although the Integrator–PP2A complex is a clear exception). Moreover, the pervasive role of PP2A in restraining proliferation provided a stronger impetus to PP2A targeted drug discovery. There is no such unity of function among different PP1 holoenzymes, which play disparate roles in diverse cellular pathways; it remains unclear whether drug development campaigns should aim for inhibitors or activators. Nevertheless, PP1 holoenzymes are clearly relevant in human disease, as a recent review of genetically engineered mouse models (GEMMs) of PP1 isoforms or PIPs revealed (Ferreira et al. 2019). Dozens of the GEMMs had disease-associated phenotypes. Different diseases were associated with PP1 activation or inactivation, and there was specificity in both the tissues and cellular compartments involved (Ferreira et al. 2019). Moreover, the diversity of PP1 holoenzymes augurs accessibility to pharmacophores that target less-frequented patches or pockets that constitute protein–protein interaction surfaces, rather than the catalytic centers. A recent success story that illustrates this point is the development of Raphin1, a compound specifically targeting the holoenzyme PP1-PPP1R15B (but not the highly similar PP1-PPP1R15A), which controls the phosphorylation status of the translation initiation factor eIF2α (Krzyzosiak et al. 2018).

Conclusions and perspective: kinase and phosphatase circuits and cycles in transcription

Work over nearly three decades has produced a detailed timeline of kinase functions needed in transcription, and an extensive but still growing inventory of the phosphatases with which kinases collaborate to drive the RNAPII cycle. The cell cycle is also driven by coordinated action of CDKs and opposing phosphatases and provides precedents and paradigms for studying transcription; deeper understanding will emerge as we learn how the key regulatory enzymes signal to the core machinery and to each other. Examples of CDK-CDK cross talk in transcription include a function of the Mediator-associated CDK8 in recruiting CDK7 and CDK9 to initiation or elongation complexes (Donner et al. 2010), the direct activation of CDK9 by CDK7 (Larochelle et al. 2012), and substrate "priming"—the preference of certain CDKs for the CTD previously phosphorylated by a different CDK (Viladevall et al. 2009; Czudnochowski et al. 2012; St. Amour et al. 2012; Bösken et al. 2014; Greifenberg et al. 2016; Mayfield et al. 2019).

Connections have also been uncovered between CDKs and phosphatases. Phosphorylation of CDK9 within its activation loop (T-loop) is required for full activity but also

for binding to an inhibitory 7SK-HEXIM1/2 ribonucleoprotein (RNP) complex; PP1, PP2A, and PP2B have been implicated, directly or indirectly, in regulating CDK9 T-loop phosphorylation and thereby the distribution of CDK9 between RNP-bound and RNP-free forms (Ammosova et al. 2005; Chen et al. 2008). Conversely, CDK9 places inhibitory phosphorylations on PP1 catalytic subunits in fission yeast and human cells, thereby preventing dephosphorylation of another CDK9 target, the SPT5 CTRs, and maintaining a rapid elongation rate (Sansó et al. 2016; Parua et al. 2018, 2020). A nearly identical signaling module, comprising PP1 and CDK1, controls the switch-like entry to and exit from mitosis in both fission yeast and metazoans (Grallert et al. 2015). Similarly, a CDK9–PP4 circuit duplicates one previously found to contain a cell cycle CDK and PP4 (Voss et al. 2013). Moreover, just as kinases are often arranged in linear cascades, phosphatase relays play decisive roles in cell signaling pathways; a relevant example occurs during mitotic exit, when PP1 autodephosphorylates to become active and, in turn, dephosphorylates and activates PP2A (Grallert et al. 2015). Similar phosphatase cascades are likely to emerge in the RNAPII transcription cycle, given the recently documented involvement of both PP1 (Kecman et al. 2018; Parua et al. 2018, 2020; Cortazar et al. 2019; Eaton et al. 2020) and PP2A (Huang et al. 2020; Zheng et al. 2020; Vervoort et al. 2021) and the reported interaction of PP4 and PP2A subunits in *C. elegans* (Sen et al. 2020).

Both the CDK9–PP4 and CDK9–PP1 interactions are likely to make transcription cycle transitions—between promoter-proximally paused and elongating RNAPII and between elongating and 3′ paused RNAPII, respectively—more switch-like and spatially precise (Parua et al. 2020). Moreover, a network in which different phosphatases (PP1, PP2A, and PP4) oppose the actions of a single kinase (CDK9) can achieve spatial and temporal complexity in the modification patterns it creates on multisite phosphorylation substrates such as RNAPII and SPT5, another regulatory strategy previously uncovered in the cell cycle (Swaffer et al. 2016).

An important question still to be addressed is whether phosphatase activity is truly regulatory under physiologic conditions. Transcriptional CDKs have attracted intense interest not only because of their inherent druggability, but also because of their roles in transducing extrinsic signals to the RNAPII machinery to promote or repress gene expression. For example, CDK9 can be induced by genotoxic stress or co-opted by viral or oncogenic *trans*-activators to drive specific transcription programs (Bacon and D'Orso 2019). We are not aware of a clear example of phosphatases acting in such a decisive way, but the involvement of PP1 holoenzymes in the control of RNAPII elongation rate (Parua et al. 2018, 2020; Cortazar et al. 2019; Eaton et al. 2020) suggests where we might look to find one. In yeast and metazoans, nutrient-sensing and other signaling pathways use transcriptional interference between neighboring genes as a regulatory switch (Kaikkonen and Adelman 2018; Shuman 2020). In fission yeast, key genes encoding proteins needed for organic phosphate acquisition (*pho* genes) are arranged in tandem with

lncRNA genes; under phosphate-replete conditions, read-through transcription driven by the upstream lncRNA promoters disrupts DNA binding by an activator of the downstream protein-coding genes, limiting their expression. Upon phosphate starvation, the lncRNA transcripts terminate farther upstream, and expression of the *pho* genes—*pho1*$^+$, *pho84*$^+$, and *tgp1*$^+$—is induced (Shuman 2020). This control can be disrupted by a mutation in *rpb1* that decreases the intrinsic rate of elongation by RNAPII, favoring termination of lncRNA transcripts near a more upstream PAS and derepressing *tgp1*$^+$ under phosphate-replete conditions (Yague-Sanz et al. 2020). Conversely, hyperrepression of *pho* genes was elicited by mutations that impair 3′ end processing or termination: a CTD-T4A substitution in the *rpb1* gene, and mutations in subunits of CPF, including *ssu72* and *ppn1* (Sanchez et al. 2018). In contrast to the slow *rpb1* mutant described above, loss of CPF-associated PP1 is likely to attenuate or delay the deceleration of RNAPII that promotes termination downstream from the PAS (Kecman et al. 2018; Parua et al. 2018), possibly contributing to *pho* gene hyperrepression. The choice of a lncRNA 3′ end processing site is responsive to cellular levels of specific inositol pyrophosphates (IPPs) (Sanchez et al. 2019; Garg et al. 2020); it will be interesting to map the downstream targets of this signaling, which might include transcriptional kinases, phosphatases, or both.

It is a safe bet that more regulatory circuits, involving other kinases and phosphatases, will emerge in the next few years. We predict the traffic will flow both ways, with phosphatases also signaling to kinases, and include all permutations of negative and positive regulation. An intriguing question for the future is how phosphatases coordinate with the CDKs to regulate liquid–liquid phase separation as RNAPII transits in and out of condensates with different proteins (Cramer 2019b). Phosphorylation by CDKs can dissolve transcription "hubs" containing RNAPII and PIC components and facilitate formation of distinct condensates containing RNAPII and splicing factors (Boehning et al. 2018; Lu et al. 2018; Guo et al. 2019). It seems nearly inevitable that phosphatases will play complementary roles as "cogatekeepers" controlling ingress and egress at these assemblies. Given the rapid recent progress in the field, moreover, a final, seemingly safe prediction is that there will be more surprises, and new players enlisted—including other, "famous" phosphatases such as the tumor suppressor PTEN (Steinbach et al. 2019)—in a truly comprehensive model of the RNAPII transcription cycle.

Competing interest statement

The authors declare no competing interests.

Acknowledgments

References

Adelman K, Lis JT. 2012. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat Rev Genet* **13:** 720–731. doi:10.1038/nrg3293

Ammosova T, Washington K, Debebe Z, Brady J, Nekhai S. 2005. Dephosphorylation of CDK9 by protein phosphatase 2A and protein phosphatase-1 in Tat-activated HIV-1 transcription. *Retrovirology* **2:** 47. doi:10.1186/1742-4690-2-47

Ansari A, Hampsey M. 2005. A role for the CPF 3′-end processing machinery in RNAP II-dependent gene looping. *Genes Dev* **19:** 2969–2978. doi:10.1101/gad.1362305

Archambault J, Chambers RS, Kobor MS, Ho Y, Cartier M, Bolotin D, Andrews B, Kane CM, Greenblatt J. 1997. An essential component of a C-terminal domain phosphatase that interacts with transcription factor IIF in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci* **94:** 14300–14305. doi:10.1073/pnas.94.26 .14300

Archambault J, Pan G, Dahmus GK, Cartier M, Marshall N, Zhang S, Dahmus ME, Greenblatt J. 1998. FCP1, the RAP74-interacting subunit of a human protein phosphatase that dephosphorylates the carboxyl-terminal domain of RNA polymerase IIO. *J Biol Chem* **273:** 27593–27601. doi:10.1074/jbc .273.42.27593

Austenaa LM, Barozzi I, Simonatto M, Masella S, Della Chiara G, Ghisletti S, Curina A, de Wit E, Bouwman BA, de Pretis S, et al. 2015. Transcription of mammalian *cis*-regulatory elements is restrained by actively enforced early termination. *Mol Cell* **60:** 460–474. doi:10.1016/j.molcel.2015.09.018

Bacon CW, D'Orso I. 2019. CDK9: a signaling hub for transcriptional control. *Transcription* **10:** 57–75. doi:10.1080/ 21541264.2018.1523668

Bataille AR, Jeronimo C, Jacques PE, Laramée L, Fortin ME, Forest A, Bergeron M, Hanes SD, Robert F. 2012. A universal RNA polymerase II CTD cycle is orchestrated by complex interplays between kinase, phosphatase, and isomerase enzymes along genes. *Mol Cell* **45:** 158–170. doi:10.1016/j .molcel.2011.11.024

Beltrao P, Bork P, Krogan NJ, van Noort V. 2013. Evolution and functional cross-talk of protein post-translational modifications. *Mol Syst Biol* **9:** 714. doi:10.1002/msb.201304521

Bertolotti A. 2018. The split protein phosphatase system. *Biochem J* **475:** 3707–3723. doi:10.1042/BCJ20170726

Boehning M, Dugast-Darzacq C, Rankovic M, Hansen AS, Yu T, Marie-Nelly H, McSwiggen DT, Kokic G, Dailey GM, Cramer P, et al. 2018. RNA polymerase II clustering through carboxy-terminal domain phase separation. *Nat Struct Mol Biol* **25:** 833–840. doi:10.1038/s41594-018-0112-y

Booth GT, Wang IX, Cheung VG, Lis JT. 2016. Divergence of a conserved elongation factor and transcription regulation in budding and fission yeast. *Genome Res* **26:** 799–811. doi:10 .1101/gr.204578.116

Booth GT, Parua PK, Sansó M, Fisher RP, Lis JT. 2018. Cdk9 regulates a promoter-proximal checkpoint to modulate RNA polymerase II elongation rate in fission yeast. *Nat Commun* **9:** 543. doi:10.1038/s41467-018-03006-4

Bösken CA, Farnung L, Hintermair C, Merzel Schachter M, Vogel-Bachmayr K, Blazek D, Anand K, Fisher RP, Eick D, Geyer M. 2014. The structure and substrate specificity of human Cdk12/Cyclin K. *Nat Commun* **5:** 3505. doi:10.1038/ ncomms4505

Bradner JE, Hnisz D, Young RA. 2017. Transcriptional addiction in cancer. *Cell* **168:** 629–643. doi:10.1016/j.cell.2016.12.013

Brannan K, Kim H, Erickson B, Glover-Cutter K, Kim S, Fong N, Kiemele L, Hansen K, Davis R, Lykke-Andersen J, et al. 2012. mRNA decapping factors and the exonuclease Xrn2 function in widespread premature termination of RNA polymerase II transcription. *Mol Cell* **46:** 311–324. doi:10.1016/j.molcel.2012.03.006

Breitkreutz A, Choi H, Sharom JR, Boucher L, Neduva V, Larsen B, Lin ZY, Breitkreutz BJ, Stark C, Liu G, et al. 2010. A global protein kinase and phosphatase interaction network in yeast. *Science* **328:** 1043–1046. doi:10.1126/science.1176495

Buratowski S. 2003. The CTD code. *Nat Struct Biol* **10:** 679–680. doi:10.1038/nsb0903-679

Buratowski S. 2009. Progression through the RNA polymerase II CTD cycle. *Mol Cell* **36:** 541–546. doi:10.1016/j.molcel.2009.10.019

Ceulemans H, Bollen M. 2004. Functional diversity of protein phosphatase-1, a cellular economizer and reset button. *Physiol Rev* **84:** 1–39. doi:10.1152/physrev.00013.2003

Chambers RS, Dahmus ME. 1994. Purification and characterization of a phosphatase from HeLa cells which dephosphorylates the C-terminal domain of RNA polymerase II. *J Biol Chem* **269:** 26243–26248. doi:10.1016/S0021-9258(18)47186-4

Chambers RS, Kane CM. 1996. Purification and characterization of an RNA polymerase II phosphatase from yeast. *J Biol Chem* **271:** 24498–24504. doi:10.1074/jbc.271.40.24498

Chambers RS, Wang BQ, Burton ZF, Dahmus ME. 1995. The activity of COOH-terminal domain phosphatase is regulated by a docking site on RNA polymerase II and by the general transcription factors IIF and IIB. *J Biol Chem* **270:** 14962–14969. doi:10.1074/jbc.270.25.14962

Chen R, Liu M, Li H, Xue Y, Ramey WN, He N, Ai N, Luo H, Zhu Y, Zhou N, et al. 2008. PP2B and PP1α cooperatively disrupt 7SK snRNP to release P-TEFb for transcription in response to Ca^{2+} signaling. *Genes Dev* **22:** 1356–1368. doi:10.1101/gad.1636008

Chen MJ, Dixon JE, Manning G. 2017. Genomics and evolution of protein phosphatases. *Sci Signal* **10:** eaag1796. doi:10.1126/scisignal.aag1796

Chipumuro E, Marco E, Christensen CL, Kwiatkowski N, Zhang T, Hatheway CM, Abraham BJ, Sharma B, Yeung C, Altabef A, et al. 2014. CDK7 inhibition suppresses super-enhancer-linked oncogenic transcription in MYCN-driven cancer. *Cell* **159:** 1126–1139. doi:10.1016/j.cell.2014.10.024

Cho H, Kim TK, Mancebo H, Lane WS, Flores O, Reinberg D. 1999. A protein phosphatase functions to recycle RNA polymerase II. *Genes Dev* **13:** 1540–1552. doi:10.1101/gad.13.12.1540

Cho EJ, Kobor MS, Kim M, Greenblatt J, Buratowski S. 2001. Opposing effects of Ctk1 kinase and Fcp1 phosphatase at Ser 2 of the RNA polymerase II C-terminal domain. *Genes Dev* **15:** 3319–3329. doi:10.1101/gad.935901

Christensen CL, Kwiatkowski N, Abraham BJ, Carretero J, Al-Shahrour F, Zhang T, Chipumuro E, Herter-Sprie GS, Akbay EA, Altabef A, et al. 2014. Targeting transcriptional addictions in small cell lung cancer with a covalent CDK7 inhibitor. *Cancer Cell* **26:** 909–922. doi:10.1016/j.ccell.2014.10.019

Ciurciu A, Duncalf L, Jonchere V, Lansdale N, Vasieva O, Glenday P, Rudenko A, Vissi E, Cobbe N, Alphey L, et al. 2013. PNUTS/PP1 regulates RNAPII-mediated gene expression and is necessary for developmental growth. *PLoS Genet* **9:** e1003885. doi:10.1371/journal.pgen.1003885

Corden JL. 1990. Tails of RNA polymerase II. *Trends Biochem Sci* **15:** 383–387. doi:10.1016/0968-0004(90)90236-5

Core L, Adelman K. 2019. Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation. *Genes Dev* **33:** 960–982. doi:10.1101/gad.325142.119

Cortazar MA, Sheridan RM, Erickson B, Fong N, Glover-Cutter K, Brannan K, Bentley DL. 2019. Control of RNA Pol II speed by PNUTS-PP1 and Spt5 dephosphorylation facilitates termination by a 'sitting duck torpedo' mechanism. *Mol Cell* **76:** 896–908.e4. doi:10.1016/j.molcel.2019.09.031

Cossa G, Roeschert I, Prinz F, Baluapuri A, Silveira Vidal R, Schülein-Völk C, Chang YC, Ade CP, Mastrobuoni G, Girard C, et al. 2020. Localized inhibition of protein phosphatase 1 by NUAK1 promotes spliceosome activity and reveals a MYC-sensitive feedback control of transcription. *Mol Cell* **77:** 1322–1339.e11. doi:10.1016/j.molcel.2020.01.008

Cramer P. 2019a. Eukaryotic transcription turns 50. *Cell* **179:** 808–812. doi:10.1016/j.cell.2019.09.018

Cramer P. 2019b. Organization and regulation of gene transcription. *Nature* **573:** 45–54. doi:10.1038/s41586-019-1517-4

Czudnochowski N, Bösken CA, Geyer M. 2012. Serine-7 but not serine-5 phosphorylation primes RNA polymerase II CTD for P-TEFb recognition. *Nat Commun* **3:** 842. doi:10.1038/ncomms1846

Descostes N, Heidemann M, Spinelli L, Schüller R, Maqbool MA, Fenouil R, Koch F, Innocenti C, Gut M, Gut I, et al. 2014. Tyrosine phosphorylation of RNA polymerase II CTD is associated with antisense promoter transcription and active enhancers in mammalian cells. *Elife* **3:** e02105. doi:10.7554/eLife.02105

Dichtl B, Blank D, Ohnacker M, Friedlein A, Roeder D, Langen H, Keller W. 2002. A role for SSU72 in balancing RNA polymerase II transcription elongation and termination. *Mol Cell* **10:** 1139–1150. doi:10.1016/S1097-2765(02)00707-4

Dingar D, Tu WB, Resetca D, Lourenco C, Tamachi A, De Melo J, Houlahan KE, Kalkat M, Chan PK, Boutros PC, et al. 2018. MYC dephosphorylation by the PP1/PNUTS phosphatase complex regulates chromatin binding and protein stability. *Nat Commun* **9:** 3502. doi:10.1038/s41467-018-05660-0

Donner AJ, Ebmeier CC, Taatjes DJ, Espinosa JM. 2010. CDK8 is a positive regulator of transcriptional elongation within the serum response network. *Nat Struct Mol Biol* **17:** 194–201. doi:10.1038/nsmb.1752

Eaton JD, Francis L, Davidson L, West S. 2020. A unified allosteric/torpedo mechanism for transcriptional termination on human protein-coding genes. *Genes Dev* **34:** 132–145. doi:10.1101/gad.332833.119

Egloff S, Murphy S. 2008. Cracking the RNA polymerase II CTD code. *Trends Genet* **24:** 280–288. doi:10.1016/j.tig.2008.03.008

Egloff S, Zaborowska J, Laitem C, Kiss T, Murphy S. 2012. Ser7 phosphorylation of the CTD recruits the RPAP2 Ser5 phosphatase to snRNA genes. *Mol Cell* **45:** 111–122. doi:10.1016/j.molcel.2011.11.006

Elrod ND, Henriques T, Huang KL, Tatomer DC, Wilusz JE, Wagner EJ, Adelman K. 2019. The integrator complex attenuates promoter-proximal transcription at protein-coding genes. *Mol Cell* **76:** 738–752.e7. doi:10.1016/j.molcel.2019.10.034

Ferreira M, Beullens M, Bollen M, Van Eynde A. 2019. Functions and therapeutic potential of protein phosphatase 1: insights from mouse genetics. *Biochim Biophys Acta Mol Cell Res* **1866:** 16–30. doi:10.1016/j.bbamcr.2018.07.019

Fuda NJ, Buckley MS, Wei W, Core LJ, Waters CT, Reinberg D, Lis JT. 2012. Fcp1 dephosphorylation of the RNA polymerase II C-terminal domain is required for efficient transcription of heat shock genes. *Mol Cell Biol* **32:** 3428–3437. doi:10.1128/MCB.00247-12

Ganem C, Devaux F, Torchet C, Jacq C, Quevillon-Cheruel S, Labesse G, Facca C, Faye G. 2003. Ssu72 is a phosphatase essential for transcription termination of snoRNAs and specific mRNAs in yeast. *EMBO J* **22:** 1588–1598. doi:10.1093/emboj/cdg141

Garg A, Shuman S, Schwer B. 2020. A genetic screen for suppressors of hyper-repression of the fission yeast PHO regulon by Pol2 CTD mutation T4A implicates inositol 1-pyrophosphates as agonists of precocious lncRNA transcription termination. *Nucleic Acids Res* **48:** 10739–10752. doi:10.1093/nar/gkaa776

Gelens L, Qian J, Bollen M, Saurin AT. 2018. The importance of kinase–phosphatase integration: lessons from mitosis. *Trends Cell Biol* **28:** 6–21. doi:10.1016/j.tcb.2017.09.005

Ghosh A, Shuman S, Lima CD. 2008. The structure of Fcp1, an essential RNA polymerase II CTD phosphatase. *Mol Cell* **32:** 478–490. doi:10.1016/j.molcel.2008.09.021

Gibney PA, Fries T, Bailer SM, Morano KA. 2008. Rtr1 is the *Saccharomyces cerevisiae* homolog of a novel family of RNA polymerase II-binding proteins. *Eukaryot Cell* **7:** 938–948. doi:10.1128/EC.00042-08

Glover-Cutter K, Larochelle S, Erickson B, Zhang C, Shokat K, Fisher RP, Bentley DL. 2009. TFIIH-associated Cdk7 kinase functions in phosphorylation of C-terminal domain Ser7 residues, promoter-proximal pausing, and termination by RNA polymerase II. *Mol Cell Biol* **29:** 5455–5464. doi:10.1128/MCB.00637-09

Grallert A, Boke E, Hagting A, Hodgson B, Connolly Y, Griffiths JR, Smith DL, Pines J, Hagan IM. 2015. A PP1-PP2A phosphatase relay controls mitotic progression. *Nature* **517:** 94–98. doi:10.1038/nature14019

Greifenberg AK, Hönig D, Pilarova K, Düster R, Bartholomeeusen K, Bösken CA, Anand K, Blazek D, Geyer M. 2016. Structural and functional analysis of the Cdk13/Cyclin K complex. *Cell Rep* **14:** 320–331. doi:10.1016/j.celrep.2015.12.025

Guo YE, Manteiga JC, Henninger JE, Sabari BR, Dall'Agnese A, Hannett NM, Spille JH, Afeyan LK, Zamudio AV, Shrinivas K, et al. 2019. Pol II phosphorylation regulates a switch between transcriptional and splicing condensates. *Nature* **572:** 543–548. doi:10.1038/s41586-019-1464-0

Heroes E, Lesage B, Görnemann J, Beullens M, Van Meervelt L, Bollen M. 2013. The PP1 binding code: a molecular-lego strategy that governs specificity. *FEBS J* **280:** 584–595. doi:10.1111/j.1742-4658.2012.08547.x

Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E. 2015. Phosphositeplus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res* **43:** D512–D520. doi:10.1093/nar/gku1267

Hsin JP, Manley JL. 2012. The RNA polymerase II CTD coordinates transcription and RNA processing. *Genes Dev* **26:** 2119–2137. doi:10.1101/gad.200303.112

Hsin JP, Xiang K, Manley JL. 2014. Function and control of RNA polymerase II C-terminal domain phosphorylation in vertebrate transcription and RNA processing. *Mol Cell Biol* **34:** 2488–2498. doi:10.1128/MCB.00181-14

Hsu PL, Yang F, Smith-Kinnaman W, Yang W, Song JE, Mosley AL, Varani G. 2014. Rtr1 is a dual specificity phosphatase that dephosphorylates Tyr1 and Ser5 on the RNA polymerase II CTD. *J Mol Biol* **426:** 2970–2981. doi:10.1016/j.jmb.2014.06.010

Huang KL, Jee D, Stein CB, Elrod ND, Henriques T, Mascibroda LG, Baillat D, Russell WK, Adelman K, Wagner EJ. 2020. Integrator recruits protein phosphatase 2A to prevent pause release and facilitate transcription termination. *Mol Cell* **80:** 345–358.e9. doi:10.1016/j.molcel.2020.08.016

Irani S, Yogesha SD, Mayfield J, Zhang M, Zhang Y, Matthews WL, Nie G, Prescott NA, Zhang YJ. 2016. Structure of *Saccharomyces cerevisiae* Rtr1 reveals an active site for an atypical phosphatase. *Sci Signal* **9:** ra24. doi:10.1126/scisignal.aad4805

Jeronimo C, Forget D, Bouchard A, Li Q, Chua G, Poitras C, Thérien C, Bergeron D, Bourassa S, Greenblatt J, et al. 2007. Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme. *Mol Cell* **27:** 262–274. doi:10.1016/j.molcel.2007.06.027

Jonkers I, Kwak H, Lis JT. 2014. Genome-wide dynamics of Pol II elongation and its interplay with promoter proximal pausing, chromatin, and exons. *Elife* **3:** e02407. doi:10.7554/eLife.02407

Kaikkonen MU, Adelman K. 2018. Emerging roles of non-coding RNA transcription. *Trends Biochem Sci* **43:** 654–667. doi:10.1016/j.tibs.2018.06.002

Kalan S, Amat R, Schachter MM, Kwiatkowski N, Abraham BJ, Liang Y, Zhang T, Olson CM, Larochelle S, Young RA, et al. 2017. Activation of the p53 transcriptional program sensitizes cancer cells to Cdk7 inhibitors. *Cell Rep* **21:** 467–481. doi:10.1016/j.celrep.2017.09.056

Kamenski T, Heilmeier S, Meinhart A, Cramer P. 2004. Structure and mechanism of RNA polymerase II CTD phosphatases. *Mol Cell* **15:** 399–407. doi:10.1016/j.molcel.2004.06.035

Kauko O, Imanishi SY, Kulesskiy E, Yetukuri L, Laajala TD, Sharma M, Pavic K, Aakula A, Rupp C, Jumppanen M, et al. 2020. Phosphoproteome and drug-response effects mediated by the three protein phosphatase 2A inhibitor proteins CIP2A, SET, and PME-1. *J Biol Chem* **295:** 4194–4211. doi:10.1074/jbc.RA119.011265

Kecman T, Kuś K, Heo DH, Duckett K, Birot A, Liberatori S, Mohammed S, Geis-Asteggiante L, Robinson CV, Vasiljeva L. 2018. Elongation/termination factor exchange mediated by PP1 phosphatase orchestrates transcription termination. *Cell Rep* **25:** 259–269.e5. doi:10.1016/j.celrep.2018.09.007

Kim H, Erickson B, Luo W, Seward D, Graber JH, Pollock DD, Megee PC, Bentley DL. 2010. Gene-specific RNA polymerase II phosphorylation and the CTD code. *Nat Struct Mol Biol* **17:** 1279–1286. doi:10.1038/nsmb.1913

Kobor MS, Archambault J, Lester W, Holstege FC, Gileadi O, Jansma DB, Jennings EG, Kouyoumdjian F, Davidson AR, Young RA, et al. 1999. An unusual eukaryotic protein phosphatase required for transcription by RNA polymerase II and CTD dephosphorylation in *S. cerevisiae*. *Mol Cell* **4:** 55–62. doi:10.1016/S1097-2765(00)80187-2

Kobor MS, Simon LD, Omichinski J, Zhong G, Archambault J, Greenblatt J. 2000. A motif shared by TFIIF and TFIIB mediates their interaction with the RNA polymerase II carboxy-terminal domain phosphatase Fcp1p in *Saccharomyces cerevisiae*. *Mol Cell Biol* **20:** 7438–7449. doi:10.1128/MCB.20.20.7438-7449.2000

Krishnamurthy S, He X, Reyes-Reyes M, Moore C, Hampsey M. 2004. Ssu72 Is an RNA polymerase II CTD phosphatase. *Mol Cell* **14:** 387–394. doi:10.1016/S1097-2765(04)00235-7

Krzyzosiak A, Sigurdardottir A, Luh L, Carrara M, Das I, Schneider K, Bertolotti A. 2018. Target-based discovery of an inhibitor of the regulatory phosphatase PPP1R15B. *Cell* **174:** 1216–1228.e19. doi:10.1016/j.cell.2018.06.030

Kwiatkowski N, Zhang T, Rahl PB, Abraham BJ, Reddy J, Ficarro SB, Dastur A, Amzallag A, Ramaswamy S, Tesar B, et al. 2014. Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. *Nature* **511:** 616–620. doi:10.1038/nature13393

Landsverk HB, Sandquist LE, Bay LTE, Steurer B, Campsteijn C, Landsverk OJB, Marteijn JA, Petermann E, Trinkle-Mulcahy L, Syljuåsen RG. 2020. WDR82/PNUTS-PP1 prevents transcription-replication conflicts by promoting RNA polymerase II degradation on chromatin. *Cell Rep* **33:** 108469. doi:10.1016/j.celrep.2020.108469

Larochelle S, Batliner J, Gamble MJ, Barboza NM, Kraybill BC, Blethrow JD, Shokat KM, Fisher RP. 2006. Dichotomous but stringent substrate selection by the dual-function Cdk7 complex revealed by chemical genetics. *Nat Struct Mol Biol* **13:** 55–62. doi:10.1038/nsmb1028

Larochelle S, Amat R, Glover-Cutter K, Sansó M, Zhang C, Allen JJ, Shokat KM, Bentley DL, Fisher RP. 2012. Cyclin-dependent kinase control of the initiation-to-elongation switch of RNA polymerase II. *Nat Struct Mol Biol* **19:** 1108–1115. doi:10.1038/nsmb.2399

Leonard D, Huang W, Izadmehr S, O'Connor CM, Wiredja DD, Wang Z, Zaware N, Chen Y, Schlatzer DM, Kiselar J, et al. 2020. Selective PP2A enhancement through biased heterotrimer stabilization. *Cell* **181:** 688–701.e16. doi:10.1016/j.cell.2020.03.038

Lu H, Yu D, Hansen AS, Ganguly S, Liu R, Heckert A, Darzacq X, Zhou Q. 2018. Phase-separation mechanism for C-terminal hyperphosphorylation of RNA polymerase II. *Nature* **558:** 318–323. doi:10.1038/s41586-018-0174-3

Luse DS, Parida M, Spector BM, Nilson KA, Price DH. 2020. A unified view of the sequence and functional organization of the human RNA polymerase II promoter. *Nucleic Acids Res* **48:** 7767–7785. doi:10.1093/nar/gkaa531

Malumbres M. 2014. Cyclin-dependent kinases. *Genome Biol* **15:** 122. doi:10.1186/gb4184

Mandal SS, Cho H, Kim S, Cabane K, Reinberg D. 2002. FCP1, a phosphatase specific for the heptapeptide repeat of the largest subunit of RNA polymerase II, stimulates transcription elongation. *Mol Cell Biol* **22:** 7543–7552. doi:10.1128/MCB.22.21.7543-7552.2002

Manke IA, Lowery DM, Nguyen A, Yaffe MB. 2003. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. *Science* **302:** 636–639. doi:10.1126/science.1088877

Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. 2002. The protein kinase complement of the human genome. *Science* **298:** 1912–1934. doi:10.1126/science.1075762

Mayer A, Lidschreiber M, Siebert M, Leike K, Söding J, Cramer P. 2010. Uniform transitions of the general RNA polymerase II transcription complex. *Nat Struct Mol Biol* **17:** 1272–1278. doi:10.1038/nsmb.1903

Mayfield JE, Burkholder NT, Zhang YJ. 2016. Dephosphorylating eukaryotic RNA polymerase II. *Biochim Biophys Acta* **1864:** 372–387. doi:10.1016/j.bbapap.2016.01.007

Mayfield JE, Irani S, Escobar EE, Zhang Z, Burkholder NT, Robinson MR, Mehaffey MR, Sipe SN, Yang W, Prescott NA, et al. 2019. Tyr1 phosphorylation promotes phosphorylation of Ser2 on the C-terminal domain of eukaryotic RNA polymerase II by P-TEFb. *Elife* **8:** e48725. doi:10.7554/eLife.48725

Meinhart A, Silberzahn T, Cramer P. 2003. The mRNA transcription/processing factor Ssu72 is a potential tyrosine phosphatase. *J Biol Chem* **278:** 15917–15921. doi:10.1074/jbc.M301643200

Minzel W, Venkatachalam A, Fink A, Hung E, Brachya G, Burstain I, Shaham M, Rivlin A, Omer I, Zinger A, et al. 2018. Small molecules co-targeting CKIα and the transcriptional kinases CDK7/9 control AML in preclinical models. *Cell* **175:** 171–185.e25. doi:10.1016/j.cell.2018.07.045

Moorhead GB, Trinkle-Mulcahy L, Nimick M, De Wever V, Campbell DG, Gourlay R, Lam YW, Lamond AI. 2008. Displacement affinity chromatography of protein phosphatase one (PP1) complexes. *BMC Biochem* **9:** 28. doi:10.1186/1471-2091-9-28

Morita K, He S, Nowak RP, Wang J, Zimmerman MW, Fu C, Durbin AD, Martel MW, Prutsch N, Gray NS, et al. 2020. Allosteric activators of protein phosphatase 2A display broad antitumor activity mediated by dephosphorylation of MYBL2. *Cell* **181:** 702–715.e20. doi:10.1016/j.cell.2020.03.051

Morrison DK, Murakami MS, Cleghon V. 2000. Protein kinases and phosphatases in the *Drosophila* genome. *J Cell Biol* **150:** F57–F62. doi:10.1083/jcb.150.2.F57

Mosley AL, Pattenden SG, Carey M, Venkatesh S, Gilmore JM, Florens L, Workman JL, Washburn MP. 2009. Rtr1 is a CTD phosphatase that regulates RNA polymerase II during the transition from serine 5 to serine 2 phosphorylation. *Mol Cell* **34:** 168–178. doi:10.1016/j.molcel.2009.02.025

Nedea E, He X, Kim M, Pootoolal J, Zhong G, Canadien V, Hughes T, Buratowski S, Moore CL, Greenblatt J. 2003. Organization and function of APT, a subcomplex of the yeast cleavage and polyadenylation factor involved in the formation of mRNA and small nucleolar RNA 3′-ends. *J Biol Chem* **278:** 33000–33010. doi:10.1074/jbc.M304454200

Nedea E, Nalbant D, Xia D, Theoharis NT, Suter B, Richardson CJ, Tatchell K, Kislinger T, Greenblatt JF, Nagy PL. 2008. The Glc7 phosphatase subunit of the cleavage and polyadenylation factor is essential for transcription termination on snoRNA genes. *Mol Cell* **29:** 577–587. doi:10.1016/j.molcel.2007.12.031

Nesti E, Corson GM, McCleskey M, Oyer JA, Mandel G. 2014. C-terminal domain small phosphatase 1 and MAP kinase reciprocally control REST stability and neuronal differentiation. *Proc Natl Acad Sci* **111:** E3929–E3936. doi:10.1073/pnas.1414770111

Ni Z, Xu C, Guo X, Hunter GO, Kuznetsova OV, Tempel W, Marcon E, Zhong G, Guo H, Kuo WW, et al. 2014. RPRD1A and RPRD1B are human RNA polymerase II C-terminal domain scaffolds for Ser5 dephosphorylation. *Nat Struct Mol Biol* **21:** 686–695. doi:10.1038/nsmb.2853

O'Connor CM, Perl A, Leonard D, Sangodkar J, Narla G. 2018. Therapeutic targeting of PP2A. *Int J Biochem Cell Biol* **96:** 182–193. doi:10.1016/j.biocel.2017.10.008

Ohkura H, Kinoshita N, Miyatani S, Toda T, Yanagida M. 1989. The fission yeast dis2⁺ gene required for chromosome disjoining encodes one of two putative type 1 protein phosphatases. *Cell* **57:** 997–1007. doi:10.1016/0092-8674(89)90338-3

Olson CM, Liang Y, Leggett A, Park WD, Li L, Mills CE, Elsarrag SZ, Ficarro SB, Zhang T, Düster R, et al. 2019. Development of a selective CDK7 covalent inhibitor reveals predominant cell-cycle phenotype. *Cell Chem Biol* **26:** 792–803.e10. doi:10.1016/j.chembiol.2019.02.012

Örd M, Loog M. 2019. How the cell cycle clock ticks. *Mol Biol Cell* **30:** 169–172. doi:10.1091/mbc.E18-05-0272

Parua PK, Fisher RP. 2020. Dissecting the Pol II transcription cycle and derailing cancer with CDK inhibitors. *Nat Chem Biol* **16:** 716–724. doi:10.1038/s41589-020-0563-4

Parua PK, Booth GT, Sansó M, Benjamin B, Tanny JC, Lis JT, Fisher RP. 2018. A Cdk9–PP1 switch regulates the elongation–termination transition of RNA polymerase II. *Nature* **558:** 460–464. doi:10.1038/s41586-018-0214-z

Parua PK, Kalan S, Benjamin B, Sansó M, Fisher RP. 2020. Distinct Cdk9-phosphatase switches act at the beginning and end of elongation by RNA polymerase II. *Nat Commun* **11:** 4338. doi:10.1038/s41467-020-18173-6

Pons T, Paramonov I, Boullosa C, Ibáñez K, Rojas AM, Valencia A. 2014. A common structural scaffold in CTD phosphatases that supports distinct catalytic mechanisms. *Proteins* **82:** 103–118. doi:10.1002/prot.24376

Poss ZC, Ebmeier CC, Odell AT, Tangpeerachaikul A, Lee T, Pelish HE, Shair MD, Dowell RD, Old WM, Taatjes DJ. 2016. Identification of mediator kinase substrates in human cells using cortistatin A and quantitative phosphoproteomics. *Cell Rep* **15:** 436–450. doi:10.1016/j.celrep.2016.03.030

Reyes-Reyes M, Hampsey M. 2007. Role for the Ssu72 C-terminal domain phosphatase in RNA polymerase II transcription elongation. *Mol Cell Biol* **27:** 926–936. doi:10.1128/MCB.01361-06

Rimel JK, Poss ZC, Erickson B, Maas ZL, Ebmeier CC, Johnson JL, Decker TM, Yaron TM, Bradley MJ, Hamman KB, et al. 2020. Selective inhibition of CDK7 reveals high-confidence targets and new models for TFIIH function in transcription. *Genes Dev* **34:** 1452–1473. doi:10.1101/gad.341545.120

Sanchez AM, Shuman S, Schwer B. 2018. RNA polymerase II CTD interactome with 3′ processing and termination factors in fission yeast and its impact on phosphate homeostasis. *Proc Natl Acad Sci* **115:** E10652–E10661. doi:10.1073/pnas.1810711115

Sanchez AM, Garg A, Shuman S, Schwer B. 2019. Inositol pyrophosphates impact phosphate homeostasis via modulation of RNA 3′ processing and transcription termination. *Nucleic Acids Res* **47:** 8452–8469. doi:10.1093/nar/gkz567

Sangodkar J, Farrington CC, McClinch K, Galsky MD, Kastrinsky DB, Narla G. 2016. All roads lead to PP2A: exploiting the therapeutic potential of this phosphatase. *FEBS J* **283:** 1004–1024. doi:10.1111/febs.13573

Sangodkar J, Perl A, Tohme R, Kiselar J, Kastrinsky DB, Zaware N, Izadmehr S, Mazhar S, Wiredja DD, O'Connor CM, et al. 2017. Activation of tumor suppressor protein PP2A inhibits KRAS-driven tumor growth. *J Clin Invest* **127:** 2081–2090. doi:10.1172/JCI89548

Sansó M, Fisher RP. 2013. Pause, play, repeat: CDKs push RNAP II's buttons. *Transcription* **4:** 146–152. doi:10.4161/trns.25146

Sansó M, Levin RS, Lipp JJ, Wang VY, Greifenberg AK, Quezada EM, Ali A, Ghosh A, Larochelle S, Rana TM, et al. 2016. P-TEFb regulation of transcription termination factor Xrn2 revealed by a chemical genetic screen for Cdk9 substrates. *Genes Dev* **30:** 117–131. doi:10.1101/gad.269589.115

Sansó M, Parua PK, Pinto D, Svensson JP, Page V, Bitton DA, MacKinnon S, Garcia P, Hidalgo E, Bahler J, et al. 2020. Cdk9 and H2Bub1 signal to Clr6-CII/Rpd3S to suppress aberrant antisense transcription. *Nucleic Acids Res* **48:** 7154–7168.

Schier AC, Taatjes DJ. 2020. Structure and mechanism of the RNA polymerase II transcription machinery. *Genes Dev* **34:** 465–488. doi:10.1101/gad.335679.119

Schreieck A, Easter AD, Etzold S, Wiederhold K, Lidschreiber M, Cramer P, Passmore LA. 2014. RNA polymerase II termination involves C-terminal-domain tyrosine dephosphorylation by CPF subunit Glc7. *Nat Struct Mol Biol* **21:** 175–179. doi:10.1038/nsmb.2753

Schroeder SC, Schwer B, Shuman S, Bentley D. 2000. Dynamic association of capping enzymes with transcribing RNA polymerase II. *Genes Dev* **14:** 2435–2440. doi:10.1101/gad.836300

Schweighofer A, Meskiene I. 2015. Phosphatases in plants. *Methods Mol Biol* **1306:** 25–46. doi:10.1007/978-1-4939-2648-0_2

Schwer B, Shuman S. 2011. Deciphering the RNA polymerase II CTD code in fission yeast. *Mol Cell* **43:** 311–318. doi:10.1016/j.molcel.2011.05.024

Schwer B, Ghosh A, Sanchez AM, Lima CD, Shuman S. 2015. Genetic and structural analysis of the essential fission yeast RNA polymerase II CTD phosphatase Fcp1. *RNA* **21:** 1135–1146. doi:10.1261/rna.050286.115

Sen I, Zhou X, Chernobrovkin A, Puerta-Cavanzo N, Kanno T, Salignon J, Stoehr A, Lin XX, Baskaner B, Brandenburg S, et al. 2020. DAF-16/FOXO requires protein phosphatase 4 to initiate transcription of stress resistance and longevity promoting genes. *Nat Commun* **11:** 138. doi:10.1038/s41467-019-13931-7

Shetty A, Kallgren SP, Demel C, Maier KC, Spatt D, Alver BH, Cramer P, Park PJ, Winston F. 2017. Spt5 plays vital roles in the control of sense and antisense transcription elongation. *Mol Cell* **66:** 77–88.e5. doi:10.1016/j.molcel.2017.02.023

Shi Y. 2009. Serine/threonine phosphatases: mechanism through structure. *Cell* **139:** 468–484. doi:10.1016/j.cell.2009.10.006

Shuman S. 2020. Transcriptional interference at tandem lncRNA and protein-coding genes: an emerging theme in regulation of cellular nutrient homeostasis. *Nucleic Acids Res* **48:** 8243–8254. doi:10.1093/nar/gkaa630

Sims RJ 3rd, Rojas LA, Beck DB, Bonasio R, Schuller R, Drury WJ 3rd, Eick D, Reinberg D. 2011. The C-terminal domain of RNA polymerase II is modified by site-specific methylation. *Science* **332:** 99–103. doi:10.1126/science.1202663

Smoly I, Shemesh N, Ziv-Ukelson M, Ben-Zvi A, Yeger-Lotem E. 2017. An asymmetrically balanced organization of kinases versus phosphatases across eukaryotes determines their distinct impacts. *PLoS Comput Biol* **13:** e1005221. doi:10.1371/journal.pcbi.1005221

St. Amour CV, Sansó M, Bösken CA, Lee KM, Larochelle S, Zhang C, Shokat KM, Geyer M, Fisher RP. 2012. 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* **32:** 2372–2383. doi:10.1128/MCB.06657-11

Steinbach N, Hasson D, Mathur D, Stratikopoulos EE, Sachidanandam R, Bernstein E, Parsons RE. 2019. PTEN interacts with the transcription machinery on chromatin and regulates RNA polymerase II-mediated transcription. *Nucleic Acids Res* **47:** 5573–5586. doi:10.1093/nar/gkz272

Sun ZW, Hampsey M. 1996. Synthetic enhancement of a TFIIB defect by a mutation in SSU72, an essential yeast gene encoding a novel protein that affects transcription start site selection in vivo. *Mol Cell Biol* **16:** 1557–1566. doi:10.1128/MCB.16.4.1557

Swaffer MP, Jones AW, Flynn HR, Snijders AP, Nurse P. 2016. CDK substrate phosphorylation and ordering the cell cycle. *Cell* **167:** 1750–1761 e1716. doi:10.1016/j.cell.2016.11.034

Tan-Wong SM, Zaugg JB, Camblong J, Xu Z, Zhang DW, Mischo HE, Ansari AZ, Luscombe NM, Steinmetz LM, Proudfoot NJ. 2012. Gene loops enhance transcriptional directionality. *Science* **338:** 671–675. doi:10.1126/science.1224350

Tatomer DC, Elrod ND, Liang D, Xiao MS, Jiang JZ, Jonathan M, Huang KL, Wagner EJ, Cherry S, Wilusz JE. 2019. The integrator complex cleaves nascent mRNAs to attenuate transcription. *Genes Dev* **33:** 1525–1538. doi:10.1101/gad.330167.119

Tietjen JR, Zhang DW, Rodríguez-Molina JB, White BE, Akhtar MS, Heidemann M, Li X, Chapman RD, Shokat K, Keles S, et al. 2010. Chemical-genomic dissection of the CTD code. *Nat Struct Mol Biol* **17:** 1154–1161. doi:10.1038/nsmb.1900

Vagnarelli P, Alessi DR. 2018. PP1 phosphatase complexes: undruggable no longer. *Cell* **174:** 1049–1051. doi:10.1016/j.cell.2018.08.007

Vanoosthuyse V, Legros P, van der Sar SJ, Yvert G, Toda K, Le Bihan T, Watanabe Y, Hardwick K, Bernard P. 2014. CPF-associated phosphatase activity opposes condensin-mediated

chromosome condensation. *PLoS Genet* **10:** e1004415. doi:10.1371/journal.pgen.1004415

Verbinnen I, Ferreira M, Bollen M. 2017. Biogenesis and activity regulation of protein phosphatase 1. *Biochem Soc Trans* **45:** 89–99. doi:10.1042/BST20160154

Vervoort SJ, Welsh SA, Devlin JR, Barbieri E, Knight DA, Offley S, Bjelosevic S, Costacurta M, Todorovski I, Kearney CJ, et al. 2021. The PP2A-Integrator-CDK9 axis fine-tunes transcription and can be targeted therapeutically in cancer. *Cell* (in press).

Victorino JF, Fox MJ, Smith-Kinnaman WR, Peck Justice SA, Burriss KH, Boyd AK, Zimmerly MA, Chan RR, Hunter GO, Liu Y, et al. 2020. RNA polymerase II CTD phosphatase Rtr1 fine-tunes transcription termination. *PLoS Genet* **16:** e1008317. doi:10.1371/journal.pgen.1008317

Viladevall L, St Amour CV, Rosebrock A, Schneider S, Zhang C, Allen JJ, Shokat KM, Schwer B, Leatherwood JK, Fisher RP. 2009. TFIIH and P-TEFb coordinate transcription with capping enzyme recruitment at specific genes in fission yeast. *Mol Cell* **33:** 738–751. doi:10.1016/j.molcel.2009.01.029

Virshup DM, Shenolikar S. 2009. From promiscuity to precision: protein phosphatases get a makeover. *Mol Cell* **33:** 537–545. doi:10.1016/j.molcel.2009.02.015

Vos SM, Farnung L, Boehning M, Wigge C, Linden A, Urlaub H, Cramer P. 2018a. Structure of activated transcription complex Pol II-DSIF-PAF-SPT6. *Nature* **560:** 607–612. doi:10.1038/s41586-018-0440-4

Vos SM, Farnung L, Urlaub H, Cramer P. 2018b. Structure of paused transcription complex Pol II-DSIF-NELF. *Nature* **560:** 601–606. doi:10.1038/s41586-018-0442-2

Voss M, Campbell K, Saranzewa N, Campbell DG, Hastie CJ, Peggie MW, Martin-Granados C, Prescott AR, Cohen PT. 2013. Protein phosphatase 4 is phosphorylated and inactivated by Cdk in response to spindle toxins and interacts with γ-tubulin. *Cell Cycle* **12:** 2876–2887. doi:10.4161/cc.25919

Wang Y, Zhang T, Kwiatkowski N, Abraham BJ, Lee TI, Xie S, Yuzugullu H, Von T, Li H, Lin Z, et al. 2015. CDK7-dependent transcriptional addiction in triple-negative breast cancer. *Cell* **163:** 174–186. doi:10.1016/j.cell.2015.08.063

Westermarck J, Neel BG. 2020. Piecing together a broken tumor suppressor phosphatase for cancer therapy. *Cell* **181:** 514–517. doi:10.1016/j.cell.2020.04.005

Wu P, Nielsen TE, Clausen MH. 2015. FDA-approved small-molecule kinase inhibitors. *Trends Pharmacol Sci* **36:** 422–439. doi:10.1016/j.tips.2015.04.005

Wu D, De Wever V, Derua R, Winkler C, Beullens M, Van Eynde A, Bollen M. 2018. A substrate-trapping strategy for protein phosphatase PP1 holoenzymes using hypoactive subunit fusions. *J Biol Chem* **293:** 15152–15162. doi:10.1074/jbc.RA118.004132

Xiang K, Manley JL, Tong L. 2012. The yeast regulator of transcription protein Rtr1 lacks an active site and phosphatase activity. *Nat Commun* **3:** 946. doi:10.1038/ncomms1947

Xue Y, Ouyang K, Huang J, Zhou Y, Ouyang H, Li H, Wang G, Wu Q, Wei C, Bi Y, et al. 2013. Direct conversion of fibroblasts to neurons by reprogramming PTB-regulated microRNA circuits. *Cell* **152:** 82–96. doi:10.1016/j.cell.2012.11.045

Yague-Sanz C, Vanrobaeys Y, Fernandez R, Duval M, Larochelle M, Beaudoin J, Berro J, Labbé S, Jacques PE, Bachand F. 2020. Nutrient-dependent control of RNA polymerase II elongation rate regulates specific gene expression programs by alternative polyadenylation. *Genes Dev* **34:** 883–897. doi:10.1101/gad.337212.120

Yamada T, Yamaguchi Y, Inukai N, Okamoto S, Mura T, Handa H. 2006. P-TEFb-mediated phosphorylation of hSpt5 C-terminal repeats is critical for processive transcription elongation. *Mol Cell* **21:** 227–237. doi:10.1016/j.molcel.2005.11.024

Yeo M, Lin PS, Dahmus ME, Gill GN. 2003. A novel RNA polymerase II C-terminal domain phosphatase that preferentially dephosphorylates serine 5. *J Biol Chem* **278:** 26078–26085. doi:10.1074/jbc.M301791200

Yeo M, Lee SK, Lee B, Ruiz EC, Pfaff SL, Gill GN. 2005. Small CTD phosphatases function in silencing neuronal gene expression. *Science* **307:** 596–600. doi:10.1126/science.1100801

Zhang DW, Mosley AL, Ramisetty SR, Rodríguez-Molina JB, Washburn MP, Ansari AZ. 2012. Ssu72 phosphatase-dependent erasure of phospho-Ser7 marks on the RNA polymerase II C-terminal domain is essential for viability and transcription termination. *J Biol Chem* **287:** 8541–8551. doi:10.1074/jbc.M111.335687

Zheng H, Qi Y, Hu S, Cao X, Xu C, Yin Z, Chen X, Li Y, Liu W, Li J, et al. 2020. Identification of integrator-PP2A complex (INTAC), an RNA polymerase II phosphatase. *Science* **370:** eabb5872. doi:10.1126/science.abb5872