RNA Polymerase II transcription elongation and Pol II CTD Ser2 phosphorylation
A tail of two kinases

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Abbreviations: Pol II, RNA Polymerase II; CTD, carboxy terminal domain, referring to the Pol II CTD; Ser, serine; Ser2-P, Pol II CTD serine 2 phosphorylation; Ctk, carboxy terminal kinase, Ctk1/2/3 is a Ser2 kinase complex; Bur, bypasses upstream kinase, Cdk9, 12, and 13 are Ser2 kinases; Spt, suppressor of Ty’s, Spr4/5 makes up the DSIF transcription elongation complex; DSIF, DRB sensitive inhibitory factor; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, transcription elongation inhibitor; NELF, negative elongation factor; P-TEF, positive transcription elongation factor, P-TEFb is composed of Cdk9 and Cyclin T

The transition between initiation and productive elongation during RNA Polymerase II (Pol II) transcription is a well-appreciated point of regulation across many eukaryotes. Elongating Pol II is modified by phosphorylation of serine 2 (Ser2) on its carboxy terminal domain (CTD) by two kinases, Bur1/Ctk1 in yeast and Cdk9/Cdk12 in metazoans. Here, we discuss the roles and regulation of these kinases and their relationship to Pol II elongation control, and focus on recent data from work in C. elegans that point out gaps in our current understand of transcription elongation.

Introduction

The eukaryote RNA polymerase II (Pol II) holoenzyme is a 12-subunit complex that transcribes protein coding genes and many non-coding RNA (ncRNA) genes. Pol II activity is tightly regulated at three distinct phases: (1) initiation, which includes transcription start site selection, formation of the open complex, and production of the first few phosphodiester bonds in the RNA transcript; (2) elongation, or progression of RNA polymerase through a locus as it lengthens the RNA transcript; and (3) termination, or the release of polymerase when it reaches the end of the gene being transcribed. Recruitment of Pol II to a locus is perhaps the most conceptually straightforward mode of regulating gene expression, and thus Pol II initiation was thought to be the rate-limiting step in gene expression for many years. Indeed, today we understand the regulation of Pol II initiation by the general transcription factors (GTFs) at a detailed molecular level.1,2

During the past several years, biochemical and genomic analyses have revealed that Pol II recruitment is not the only point of transcriptional regulation. Rather, the transition between initiation and elongation is a multi-step process where any point between recruitment and productive elongation can potentially be regulated for proper gene expression in eukaryotes.3,4 Accompanying this transition is the phosphorylation of specific residues on the extended C-terminal domain (CTD) of Pol II. In this review, we will discuss the relationship between Pol II elongation and phosphorylation of one of the CTD residues, Serine 2 (Ser2), across several model systems. Although it is appreciated that this modification correlates with elongation, how Ser2 phosphorylation is regulated in a multi-cellular context and its requirement during elongation is an emerging field of interest. Recent results from studies on tissue-specific regulation of this modification in C. elegans have bearing on these questions, and are informative for how Ser2 phosphorylation, and the kinases that add this modification, are linked to Pol II elongation.

The Pol II CTD

The large subunit of Pol II, Rbp1, contains a repeated motif on the C-terminal end, called the “C terminal domain,” or Pol II CTD.5,6 A highly conserved feature of the CTD is that it is composed of dozens of repeats of the heptapeptide sequence Y1-S2-P3-T4-S5-P6-S7, (for a review see refs. 7 and 8). All residues within the CTD heptad repeat can be modified either by phosphorylation (tyrosine, threonine, serine) or isomerization (proline).5 However, serine 5 and serine 2 phosphorylation (Ser5-P and Ser2-P) are the best studied and appear to be the most conserved general marks of transcription.10 Importantly, it was recognized 14 years ago that these modifications correlate with different steps of the transcription cycle. In 2000, Buratowski and colleagues showed that Pol II phosphorylated on Ser5 is found near the 5’ end of genes and Pol II accumulates phosphorylated
Ser2 as it progresses through a gene. This has been shown to be a general phenomenon using genome-wide analysis in yeast and mammalian cells. Thus, the current model is that for the vast majority of protein-coding genes, Pol II is recruited to a gene with a hypophosphorylated CTD; the CTD then becomes phosphorylated on Ser5 during initiation, and subsequently phosphorylated on Ser2 during productive elongation (for a review see refs. 14 and 15). This differential phosphorylation of Ser2 and Ser5 functions to recruit transcription-associated proteins at the appropriate phase of the transcription cycle (for a review see refs. 9 and 14), thus acting as an important “docking station” that can be regulated for gene expression.

More recently, additional CTD residues (Tyr1, Thr4, and Ser7) have also been shown to be phosphorylated in vivo. However, these phosphorylation events either are not generally required for transcription of protein-coding genes, or their specific role in transcription is not as yet understood. In particular, Ser7 phosphorylation can be found throughout gene bodies in yeast. Despite its presence on protein-coding genes, Ser7-P is particularly important for proper transcription of snRNA genes and may or may not be required for transcription or processing of mRNAs. Thus, because Ser2 phosphorylation has the tightest association with transcription elongation regulation, we focus our discussion on the kinases that carry out this modification. More complete reviews of the additional CTD modifications are available elsewhere.

Before discussing Ser2 kinases, several caveats of current experimental approaches should be taken into consideration. A large majority of the studies on Pol II CTD kinases have been performed using antibodies thought to be specific for distinct CTD modifications. These studies are limited by the same caveats that plague other antibody studies: variations in specificity under different situations and epitope masking. For example, antibodies thought to recognize specific phosphoepitopes can be significantly influenced by the status of phosphorylation on neighboring residues. This can produce dramatic differences in the conclusions drawn about the status of specific CTD phosphoepitopes within a gene. Additionally, as both immunofluorescence and immunoprecipitation experiments often require fixation of samples, it is possible that crosslinking between the CTD and CTD-binding proteins may mask the accessibility of the CTD to antibodies. Thus, low signal from antibody studies may not arise from the lack of a CTD modification, but from masking of the epitope by a crosslinked protein that itself associates with the epitope in the cell. Finally, studies of the Pol II CTD are particularly challenging to interpret because of the repetitive nature of the CTD. Studies using antibodies against CTD modifications cannot inform how many heptad repeats are modified or which repeats on the CTD are modified. Thus, increases in signal could indicate that more Pol II molecules are phosphorylated, that more repeats of the CTD on one Pol II molecule are modified, or simply that the epitope is more accessible. Additionally, it should be noted that not all of the repeats on the CTD from any eukaryote have the conserved heptad sequence, and many eukaryotes harbor Pol II CTDs that vary quite dramatically from the consensus repeat. As an illustration, many of the yeast CTD heptads match the consensus, while only two of the Drosophila heptads perfectly match the consensus (for a review, see refs. 8 and 10). The functional differences between these distinct repeats are not well understood; however, it should be noted these different heptads can also influence antibody studies.

### Table 1. RNA Polymerase II elongation factor homologs

|         | *S. cerevisae* | *S. pombe* | *C. elegans* | *Drosophila* | *Mammals* |
|---------|----------------|------------|--------------|--------------|-----------|
| 5’ Ser2 | kinase         | Bur1       | Cdk9         | CDK-9        | CDK9/PIKARE |
|         | cyclin(s)      | Bur2       | PCh1         | CIT-1.1      | CYCT      |
| 3’ Ser2 | kinase(s)      | Ctk1       | Lsk1         | CDK-12       | CDK12     |
|         | cyclin(s)      | Ctk2       | Lsc1         | CCNK-1       | CYCK      |
|         |                | Ctk3       | Lsg1         | *           | *         |
|         |                |            |              |              |           |
|         |                | Spt4       | Spt4         | SPT-4        | SPT4/p160 |
|         |                | Spt5       | Spt5         | SPT-5        | SPT5/p14  |
|         |                | *          | *            | *            |           |
|         |                |            |              |              |           |
|         |                |            |              |              |           |
|         |                |            |              |              |           |
| DSIF    |                |            |              |              |           |
|         |                |            |              |              |           |
| NELF    |                |            |              |              |           |

*no significant homolog detected. †homologs detected in lower eukaryotes contain an RRM similar to that found in NELFE but do not appear to be functional homologs.

Eukaryotes have two kinases that phosphorylate Ser2: Bur1/CDK9 and Ctk1/Lsk1/CDK12 (Table 1). Although initially thought to have distinct functions in yeast and mammals, recent...
Carboxy terminal kinase 1, Ctk1. Ctk1 forms a complex with its cyclin partner, Ctk2, and Ctk3, which is important for stability of the complex. The other budding yeast Ser2 kinase complex components, Bur1 and Bur2, were identified in a screen for factors that could bypass the need for upstream activating sequences (UAS) of a reporter gene (BUR, bypass UAS requirement). These proteins were also found to comprise a cyclin dependent kinase complex in which Bur1 is the kinase and Bur2 is the cyclin. While genetic studies linked Bur1 activity with phosphorylation of the CTD, it was not shown to be a direct CTD kinase in vivo until several years later.

Interestingly, although Ctk1 is considered the major Ser2 kinase in S. cerevisiae, it is not an essential protein. Null mutants for Ctk1 complex components are viable and healthy at normal temperatures, but show cold sensitive phenotypes. In contrast, Bur1 is important for normal growth as both bur1 and bur2 null mutants are extremely slow growing. Similarly, in fission yeast, mutants of the Ctk1 homolog, Lsk1, are also viable under normal growth conditions, while mutants of the Bur1 homolog, Cdk9, are lethal (Fig. 1A). This suggests that the activity of Bur1 is more important for yeast growth under normal conditions than the major Ser2 kinase, Ctk1. This could be due to its role in phosphorylating other sites on the CTD such as Ser5 and Ser7 or other proteins besides Pol II including the elongation factor Spt5.

Burl1/Cdk9 and Ctk1/Lsk1 regulate yeast Ser2 phosphorylation together

Recent studies using analog sensitive mutants of Ctk1 and Bur1 established an epistatic relationship between these factors. This work showed that loss of Bur1 activity results in dramatic decreases in Ser2 phosphorylation. Additionally, the authors showed that the trace amounts (~10%) of Ser2-P detected in a ctk1 deletion strain are lost with Bur1 inhibition. Thus, while Ctk1 is responsible for the majority of Ser2-P, loss of Bur1 activity also shows significant decreases in Ser2-P. Significantly, a ctk1 mutant shows more dramatic decreases in Ser2-P at the 3′ end of a gene than the 5′ end. This suggests that the activity of Bur1 acts on Pol II at the 5′ end of genes, and this phosphorylation enhances phosphorylation by Ctk1 further downstream in the gene (Fig. 1A and B).

Fission yeast regulate Ser2-P in a similar fashion. While an overwhelming majority of detectable Ser2-P is absent with loss of activity of the Ctk1 homolog, Lsk1, recent data using analog sensitive mutants has also shown that fission yeast Cdk9 is responsible for some level of Ser2-P.

Regulation of yeast Ser2 kinases

Mirroring their roles in Ser2 phosphorylation, both Ctk1 and Bur1 are associated with elongating Pol II by chromatin immunoprecipitation (ChIP) and this has been shown genome wide for Ctk1. In yeast, these kinases appear to be recruited to loci through general mechanisms, rather than gene-specific factors. Specifically, Bur1 contains a phospho-CTD interaction domain, which interacts with Ser5-P to enhance Bur1 recruitment to genes; Ctk1 is proposed to subsequently interact with Pol II through the Bur1-phosphorylated CTD.
Because the factors involved in Ser2 kinase activity identified thus far in yeast are not gene-specific regulators, recruitment of Ser2 kinases may not play a role in the regulation of Pol II elongation, unlike in higher eukaryotes (discussed below). Instead, these kinases seem to act largely as general factors that mark elongating Pol II for the recruitment of other factors at the proper stage of transcription. Therefore, regulation of this process at individual genes may have evolved as a process in multi-cellular organisms to control spatial and temporal expression during development.

The metazoan Ser2 kinases: CDK9 and CDK12

Metazoans have well-conserved homologs of Bur1 (CDK9) and Ctk1 (CDK12). Until recently, CDK9 was thought to be the sole Ser2 kinase in metazoans. When in a complex with its cyclin partner, Cyclin T, CDK9 is referred to as positive transcription elongation factor b, or P-TEFb, as it was first identified as having an ability to enhance Pol II elongation in vitro. CDK9 is a well-studied protein, in part because of early associations of CDK9 function with HIV infection (for a review see ref. 49) and more recently because of the role of CDK9 in the regulation of Pol II elongation (discussed below). However, the role of the metazoan Ctk1 homolog, CDK12, in Ser2 phosphorylation has been more recently appreciated.

Identification of CDK9

The P-TEFb complex, CDK9/Cyclin T, was identified as a factor that positively regulates transcriptional activity and phosphorylates the CTD in Drosophila and mammalian systems (for a review see ref. 49). Although these early studies established the CTD as the major target of CDK9 within Pol II, there have been conflicting reports about the specific residues targeted by CDK9. In vitro studies suggest CDK9 may phosphorylate Ser5, Ser2, and Ser7. In contrast, in vivo studies that have shown that Ser2 phosphorylation is most affected when CDK9 is inactivated. CDK9 activity is tightly regulated, and in higher eukaryotes it is largely contained within multi-protein regulatory complexes that have been extensively characterized in Drosophila, mouse, and human. These complexes have been discussed in previous reviews (see below). Briefly, P-TEFb is negatively regulated by the 7SK/HEXIM complex (for a review see refs. 63 and 64). Additionally, P-TEFb on active genes is found in a large complex of other elongation factors, called the “Super Elongation Complex,” or SEC. Current models suggest that for P-TEFb to enhance elongation, it must be actively recruited to loci by other factors such as the Mediator proteins, CDK8, Med26, the bromodomain protein, Brd4, splicing factors, or by transcription factors, deemed “P-TEFb facilitators” (for a review see refs. 79 and 80). Some of these factors not only influence P-TEFb activity but also help release P-TEFb from the inhibitory 7SK/HEXIM complex (for a review see ref. 81).

It should be noted that Brd4 has also been identified as a Ser2 kinase in human cells; however, its role in the transcription cycle is unclear as it also regulates CDK9 activity. Furthermore, Brd4 is not present in lower eukaryotes.

CDK12/Cyclin K: the recently appreciated CTD kinase complex

The role of CDK12 in CTD phosphorylation was demonstrated in 2010 when Greenleaf and colleagues showed that it phosphorylates Ser2 in vitro and in vivo. ChIP studies revealed that CDK12 is enriched toward the 3′ end of genes, which correlates with the 3′ enrichment of Ser2-P. In addition, the authors showed that a S. cerevisiae Bur1 chimera with the CDK9 kinase domain rescues a bur1 deletion while a Ctk1/CDK12 kinase domain chimera rescues a ctk1 mutant, illustrating conservation of function. Based on these observations, as well as the fact that inhibition of CDK9 activity frequently reduces all detectable Ser2-P, Greenleaf and colleagues proposed a model for metazoan Ser2-P regulation that more closely matches Ser2-P regulation in yeast: CDK9 phosphorylates Ser2-P at the 5′ end of genes and this activity is upstream of and required for the CDK12-mediated Ser2-P toward the 3′ end of genes (discussed below, Fig. 1A and C). Therefore in both yeast and metazoans, Bur1/CDK9 acts upstream, both physically within the ORF and epistatically, of Ctk1/CDK12 to regulate Ser2 phosphorylation.

This model of Ser2 phosphorylation in higher eukaryotes is particularly attractive as it places a Ser2 kinase, CDK12, in the location of where the majority of Ser2-P is located on a gene, at the 3′ end. In many studies, CDK9 is largely located toward the 5′ end of genes while the majority of Ser2-P is found to increase toward the 3′ end of genes. While this low Ser2-P signal at CDK9 binding sites could be due to antibody detection issues discussed above, or the activity of a the Ser2 phosphatase FCP1, the identification of CDK12 as a Ser2 kinase helps to resolve this apparent paradox.

Recent studies have showed that CDK12/Cyclin K is important for the expression of long genes with a large number of exons. In particular, DNA damage response genes are down-regulated in cells with reduced CDK12 and cells lacking CDK12/Cyclin K accumulate spontaneous DNA damage. Regulation of CDK12/Cyclin K levels may also be important for differentiation. While Cyclin K levels are high in pluripotent embryonic stem cells (ESCs), they decrease through differentiation and experimentally decreasing CDK12 levels in ESCs leads to spurious differentiation. Thus, CDK12/Cyclin K is an important transcriptional regulator that needs to be more extensively characterized.

Summary of kinase requirements

It now appears that most if not all eukaryotes probably regulate Ser2 phosphorylation in a similar manner: Bur1/Cdk9 phosphorylates Ser2 toward the 5′ end of genes, and this enhances further phosphorylation by Ctk1/Cdk12 (Fig. 1B and C). Although it is satisfying that recent results have resolved two seemingly disparate modes of Ser2 regulation into one conserved model, there is one important difference between yeast and metazoans. In yeast, while Bur1 is important for Ser2 phosphorylation levels, it is not required for Ctk1-mediated phosphorylation (Fig. 1A and B). In contrast, CDK9 is essential for CDK12-mediated CTD phosphorylation in Drosophila and mammalian systems (Fig. 1A and C). The reason for...
this dissimilarity is due to differences in the regulation of transcriptional elongation between these systems.

**Pol II Elongation Control: A CDK-9-Mediated Transition in Metazoans**

Pol II pausing is pervasive in some higher eukaryotes

As mentioned in the introduction, the transitions between Pol II recruitment and productive elongation are now recognized as possible points of transcription regulation in many eukaryotes.31 One of the most general and well-documented examples of post-recruitment regulation is the transition between initiation and productive elongation.

Because many early studies of transcription were performed in yeast, where Pol II activity is largely regulated at the level of recruitment (discussed below), this was thought to be the major point of transcription regulation. However, studies in *Drosophila* and human cells reveal that higher eukaryotes exhibit substantial post-initiation regulation of transcription. Genome-wide analyses showed that at many genes, Pol II localization is highest 25–60 bp downstream of transcription start sites where Pol II “pauses” between early elongation and productive elongation.92–98 This abundance of binding is thought to reflect the fact that Pol II quickly transitions from the pre-initiation complex (PIC) to the paused state at most genes,99 and can be stably positioned at paused sites for several minutes prior to productive elongation.96,98-102 Thus, as opposed to yeast systems, the transition between transcription initiation and productive elongation is regulated in higher metazoans. While there are many ideas about the purpose of Pol II pausing, it is clear that regulating polymerase elongation allows fine-tuning of gene expression (for a discussion about proposed functions of pausing see refs. 4, 80, and 103).

**NELF is the established regulator of polymerase pausing**

Although the widespread regulation of Pol II elongation was not appreciated until recently, beautiful biochemistry by the Handa and Price labs established a framework for understanding the regulation of early transcription elongation. Specifically, this work uncovered factors that are required to mediate promoter proximal pausing: the DSIF complex (SPT4/SPT5) and the NELF complexes. DSIF interacts directly with Pol II,104 bridging contact between NELF and Pol II.105 Although the molecular details of how NELF negatively regulates Pol II is poorly understood (for a review see refs. 4 and 80), it is clear that NELF is a major mediator of pausing in systems studied to date96-108 (discussed below). Elongation repression is alleviated by P-TEFb,109,110 which, in addition to phosphorylating the Pol II CTD, also phosphorylates SPT5 of the DSIF complex111 and the NELF complex.112 Following this, NELF leaves the elongating Pol II complex while DSIF remains associated.113,114 After dissociation of NELF, DSIF acts to enhance transcription elongation111 (details below). Thus, DSIF may repress elongation simply by acting as a facilitator of NELF-mediated repression (Fig. 1C).

While the NELF complex members are only conserved in higher eukaryotes, including *Drosophila* and mammals,107 both DSIF components, SPT4 and SPT5, are highly conserved proteins (for a review see refs. 115 and 116). Studies performed in yeast, which lack NELF, suggest that DSIF acts only as a positive elongation factor,117 supporting the model that DSIF functions as a negative elongation factor in higher eukaryotes by binding NELF. In yeast, Spt5 helps to recruit transcription-associated factors such as RNA processing factors and chromatin remodelers (for a review see refs. 115 and 120-122). In addition, structural studies have determined that DSIF likely directly enhances Pol II processivity,123-125 Thus, this role of Spt5 in transcriptional regulation likely evolved early, with successive layers of regulation added throughout evolution.

Studies in other organisms also support the correlation between presence of NELF homologs and regulation of Pol II elongation. Pol II pausing has only been extensively studied in *Drosophila* and mammalian systems; however, NELF homologs are also present many other eukaryotic model systems such as *Xenopus* and *Zebrafish*. Genome-wide studies of Pol II in these systems both show a prominent 5′ accumulation of Pol II in metagene analyses.126,127 These studies suggest that elongation is a regulated event in these systems and is likely to be mediated by NELF. In contrast, *Arabidopsis* does not have obvious NELF homologs, and promoter proximal Pol II pausing has been undetected in this organism.128 In summary, evolutionary conservation of NELF correlates with widespread Pol II pausing in many eukaryotes.14

**Pol II elongation is a general step of transcription regulation in higher eukaryotes**

The roles of DSIF, NELF, and P-TEFb in pausing were first characterized using biochemical studies on naked DNA templates. This, along with the fact that these factors appear to have consistent effects on the genes tested in vitro and in vivo, led to the model that the transition of Pol II from initiation to elongation is a general point of regulation for all genes, and that these elongation factors are basal transcription factors (Fig. 1C). Genome-wide ChIP studies corroborated this conclusion as these factors are located on a surprisingly large number of genes, both active and inactive.129,130 Indeed, genes bound by DSIF and NELF significantly overlap (> 90%) with those bound by Pol II.97,131,132 Finally, functional data supports this model, as incubation of HeLa cells with flavopiridol, an inhibitor of P-TEFb, reduces the level of transcription to a level strikingly similar to incubation with α-amanitin, a well-studied Pol II inhibitor.133 Thus, P-TEFb-mediated DSIF/NELF release is a generally required and regulated step of transcription in higher eukaryotes. This explains why higher eukaryotes require CDK9 upstream of CDK12: CDK9 is required for the release from NELF-mediated pausing and subsequent elongation-associated phosphorylation of Ser2 by CDK12.

**Promoter proximal escape is not generally regulated in yeast**

Early studies in yeast established initiation as the major point of transcription regulation,134,135 and this has been confirmed for the vast majority of genes in genome-wide analyses. Pol II is fairly evenly distributed across gene bodies in budding yeast during log phase growth, suggesting there are no major rate-limiting steps in the transition between recruitment and elongation12,136 (for a
review see ref. 137). This is also the case for fission yeast, as they also lack a Pol II peak near the TSS in metagene analyses.138

Several other studies have provided more direct evidence for initiation as the major point of transcription regulation in yeast. Although recent genome-wide polymerase run-on sequencing (GRO-seq) studies in budding yeast have shown that the elongation rate can vary across a gene,119 this is not analogous to promoter proximal escape. Instead, this variation may reflect effects on Pol II processivity by the underlying DNA sequence or nucleosome positioning.119 Furthermore, changes in Pol II accumulation on a gene following a stimulus largely reflect changes in mRNA.140 Finally, genes that have initiated transcription (i.e., have Ser5 phosphorylation) highly correlate with genes where elongating Pol II is present (i.e., have Ser2 phosphorylation).12 Thus, the transition between initiation and elongation is not a common barrier to gene expression in yeast. During normal growth, genes that have initiated transcription will generally produce a full-length mRNA product.

The lack of promoter proximal pausing in yeast also correlates with Pol II Ser2 kinase requirements: Pol II elongation does not require Bur1/Cdk9 in yeast. Reducing Bur1 activity does not increase promoter proximal Pol II levels.141 Instead, there is a reduction in both initiated and elongating Pol II.139,141,142 As in budding yeast, the Bur1 homolog, Cdk9 is not required for elongation in fission yeast.143 Thus, while Bur1/Cdk9 phosphorylate the CTD toward the 5′ end of genes, is not required for the transition of Pol II into productive elongation in yeast. This is likely why Bur1/Cdk9 is not required for Ctk1/Lsk1-mediated Ser2 phosphorylation (Fig. 1A and B).

Transcriptional Elongation Regulation in C. elegans

A new facet of Ser2-P regulation and transcription elongation is now emerging in the C. elegans model system. C. elegans has been a unique model for understanding transcription regulation because it is one of the few well-studied models that has trans-splicing of Pol II transcripts. Trans-splicing, which occurs on ~70% of C. elegans genes,144,145 involves the replacement of the 5′ end of transcripts with a short, 21–22 nucleotide RNA sequence, called a splice leader.146 Because trans-splicing occurs on the majority of genes, the TSS for many Pol II genes cannot be mapped from the 5′ sequence of mature mRNA as is commonly done in other organisms.147,148 Thus, although the TSS has been mapped for individual genes in C. elegans,149 genome-wide approaches for TSS identification have only recently been performed.145,147,148,150 These recent results have, for the first time, allowed scientists to analyze the regulation of early steps of Pol II transcription on a global scale. CTD phosphorylation patterns in C. elegans are similar to other metazoans. Genome-wide studies show that Ser5-P shows stronger signal toward the promoter and Ser2 phosphorylation within the body of the gene151,152 (for a review see ref. 153). However, recent studies have identified tissue-specific regulation of Ser2 phosphorylation in C. elegans (for a review of C. elegans germline biology see refs. 154 and 155).

C. elegans Somatic Ser2-P Requires CDK-9 and CDK-12

In C. elegans embryonic somatic tissues, Ser2 phosphorylation is regulated similar to other metazoans, i.e., inhibition of CDK-9 activity results in near total loss of detectable Ser2 phosphorylation while inhibition of CDK-12 reduces Ser2 phosphorylation levels by about 60%.58,59 Additionally, CDK-9 is required for bulk transcription in C. elegans somatic tissues as loss of CDK-9 activity causes phenotypes similar to those caused by loss of embryonic RNA Pol II activity, e.g., embryonic death and defective gastrulation.59,156 Furthermore, CDK-9 activity is required for expression of somatic developmental genes in C. elegans embryos.58 Thus, in embryonic somatic tissues, as in other metazoans, it appears that CDK-9 acts upstream of CDK-12 in Ser2 phosphorylation and is required for productive transcription (Fig. 2A).

While regulation of Ser2-P in the C. elegans soma by CDK-9 parallels observations in other metazoans, there are likely distinct differences in the regulation of CDK-9 activity. For example, in higher eukaryotes, CDK9 activity is regulated by large protein complexes that include the inhibitory 7SK/HEXIM and stimulatory SEC complex. C. elegans does not contain obvious homologs for the 7SK/HEXIM components, and thus likely does not sequester CDK-9 in an analogous inhibitory complex. While C. elegans does contain homologs for a subset of the SEC components, specifically ELL1 (Y24D9A.1), AFF9 (Y55B1BR.1 and Y55B1BR.2), and EAF (D1007.16), these factors do not appear to play an essential role in transcription.157 Furthermore, knock down of ELL and AFF9 does not significantly affect either Ser2-P levels or viability (E.A. Bowman, W.G. Kelly, unpublished). Finally, whereas CDK9 activity is regulated by Brd4 and Med26 in higher eukaryotes, there is not a clear Brd4 homolog in C. elegans and Med26 does not affect bulk Ser2-P levels in somatic tissues (EA Bowman, W Kelly, unpublished).

C. elegans elongation regulation

Does C. elegans regulate Pol II promoter proximal escape?

The requirement of CDK-9 for Ser2-P in C. elegans soma suggests that CDK-9 may play a pivotal role in transcriptional elongation in this tissue. As described above, Cdk9 is required for transcription elongation in Drosophila and mammalian systems to counteract Pol II pausing mediated by NELF and DSIF. C. elegans does not have an obvious NELF complex (Table 1); however, the fact that (1) CDK-9 appears to be required for somatic transcription,58,59 (2) cells lacking CDK-9 retain a marker of initiated Pol II, Ser5-P, in the absence of the elongation marker, Ser2-P,58 and (3) Ser2-P in C. elegans somatic tissues is regulated similarly to higher metazoans suggests that Pol II elongation is still critically regulated by CDK-9 in these tissues. Is transcription elongation regulated in C. elegans and does CDK-9/P-TEFb regulate promoter proximal escape of Pol II?

Genome-wide studies show conflicting evidence for elongation regulation in C. elegans. Data from the modENCODE project using C. elegans embryos and larvae suggested that Pol II binds to genes that have low and even undetectable levels of
A separate analysis of Pol II ChIP data showed a 5′ peak of Pol II binding at both spliced and unspliced genes, although this peak can be further downstream of the TSS (100–170 bp) than paused Pol II in *Drosophila* and mammals (25–60 bp). However, using the current gold standard for identification of Pol II pausing, genome-wide polymerase run-on sequencing (GRO-seq), Meyer and colleagues did not observe evidence of wide-spread Pol II pausing in worms under optimal growth conditions.\(^{147}\)

Despite the inconsistent evidence for Pol II pausing in genome-wide analysis and averaging, some reports have suggested that pausing indeed exists at individual genes. Snyder and colleagues identified enriched Pol II promoter proximal binding at 2% of genes analyzed in normally growing embryos and larvae by ChIP-seq ("enriched" defined as 4-fold higher binding near the TSS than within the gene body; about 250 genes).\(^{159}\) Additionally, Meyer and colleagues identified several genes with a Pol II "pausing" profile in embryos and fed larvae by GRO-seq (0.38% in embryos, 15 of 3975 genes; 2.0% in larvae; "pausing" defined as TSS:body signal ratio ≥ 2).\(^{147}\)

The strongest evidence that Pol II elongation is regulated in *C. elegans* comes from studies in starved larvae. Under these conditions, Baugh and colleagues identified an accumulation of Pol II at the 5′ end of a substantial fraction of genes\(^{160}\) (for a review of larval starvation arrest see ref. 161). Most recently, two studies have confirmed that some of these genes with 5′ Pol II accumulation do indeed harbor paused Pol II. As described above, promoter proximal paused Pol II as described in *Drosophila* is transcriptionally competent rather than arrested or backtracked,\(^{96}\) and remains associated with short capped RNAs (scRNA) instead of terminating.\(^{162}\) Indeed, recent GRO-seq analysis from starved larvae showed that 7.7% of genes analyzed have a 5′ accumulation of Pol II and are transcriptionally competent.\(^{147}\) Furthermore, Baugh and colleagues showed that genes with this 5′ GRO-seq signal also produce scRNAs (short capped RNAs), which extend approximately 30–65 bp downstream of TSSs.\(^{163}\)

Furtermore, these scRNAs are longer in worms mutant for TFIIS.\(^{163}\) This lengthening of scRNAs is consistent with Pol II pausing and backtracking seen in *Drosophila*,\(^{162}\) and makes the case for Pol II pausing in *C. elegans* even more compelling. Thus, Pol II elongation is indeed regulated for a substantial number of genes in starved *C. elegans* larvae.

While the release of Pol II from initiation into elongation may not be a ubiquitous rate-limiting step during normal growth in *C. elegans*, there is strong evidence that a fraction of genes are regulated at this step and are thus "paused." At this point, it is unclear if Pol II release requires CDK-9 as is the case in *Drosophila* and mammals; however, as mentioned above CDK-9 is generally required for transcription in somatic tissues (see above). Thus, it seems likely that CDK-9 mediates promoter proximal escape at paused genes and it is attractive to consider that CDK-9 may be a general regulator of transcriptional regulation in *C. elegans*.

Possible mechanisms for *C. elegans* promoter proximal escape

Pol II elongation can be regulated during *C. elegans* transcription, and whether this is a general mechanism at all genes as in *Drosophila* and mammals, or only important for a small subset of genes, this regulation appears to occur by a mechanism that does not involve NELF. One of the biggest reasons that elongation regulation has been controversial in
C. elegans is because it lacks homologs to the NELF complex. Thus, Pol II elongation could be regulated by DSIF activity alone or via a novel, unidentified pausing factor. Importantly, C. elegans does not have clear homologs of the recently identified pausing factors Gdown1, GAGA factor, or M1BP. Can pausing be mediated by DSIF alone? DSIF is commonly referred to as a negative regulator of elongation; however, it is generally thought to perform this function in concert with NELF (discussed above). In support of a possible role for DSIF-mediated pausing, an early study showed that a heat shock gene, which requires CDK-9 for expression, is re-expressed when both CDK-9 and DSIF are knocked down in the C. elegans embryo. However, this may be unique to heat-shock regulation, or there may be additional elongation factors regulating other genes, as the authors did not observe re-expression of developmental genes when both CDK-9 and DSIF were knocked down. In summary, if CDK-9 does act to release Pol II for productive elongation in the soma, there is likely to be an additional factor that mediates this step, which may or may not work in combination with DSIF in a mechanism analogous to NELF in higher eukaryotes (Fig. 2C).

Recently, Grishok and colleagues have identified the ZFP-1 (AF10 homolog)/DOT-1 complex as a negative regulator of Pol II elongation in C. elegans. Specifically, ZFP-1 mutants cause a decrease in Pol II abundance at the promoter of several C. elegans genes. ZFP-1/DOT-1 regulates elongation by reducing histone ubiquitination that normally promotes transcription. It is unclear if this complex plays a role analogous to the NELF complex in Pol II pausing but it does suggest an interesting mode of elongation regulation through chromatin. Finally it should be noted that the ZFP-1/DOT-1 complex is not found at all genes that have a predominant 5′ Pol II accumulation, so additional factors are likely involved in this step of Pol II elongation.

Unique regulation of Ser2-P in the C. elegans germline suggests tissue-specific Pol II elongation regulation

Recent studies have uncovered tissue-specific regulation of Ser2-P in C. elegans (for a review of C. elegans germline biology see refs. 154 and 155). In contrast to its essential role in somatic Ser2-P, CDK-9 is not required for the bulk of Ser2 phosphorylation in the germline, which instead requires CDK-12. While it is unexpected that C. elegans germline Ser2-P does not require CDK-9, this pattern of Ser2-P regulation is surprisingly similar to that seen in yeast (Fig. 1A). As in yeast, Ser2 phosphorylation does not require upstream activity of the Bur1/CDK-9 kinase, but instead is largely dependent on Ctk1/CDK-12 (Fig. 2A). Additionally, in both systems, Bur1/CDK-9 is not required for bulk Ser2 phosphorylation although they are important for normal growth in each cell type. Finally, as in yeast, the Ser2 kinase, CDK-12, is also not essential for germline development in standard laboratory conditions (Fig. 2A).

Overall, Ser2-P regulation in germline tissues matches the regulation seen in yeast while Ser2-P regulation soma matches what is seen in Drosophila and mammalian systems. What is the mechanism for the differential regulation of Ser2 phosphorylation between somatic and germline tissues? As Ser2-P is most tightly associated with transcription elongation, it is appealing to think that tissue-specific Ser2-P regulation reflects differences in transcription elongation control between these two basic tissue types. While it is clear that transcription elongation can be a regulated step in C. elegans transcription (see above), it is enticing to speculate that this regulation is limited to the soma and not the germline. Perhaps somatic tissues require CDK-9 for Ser2-P because CDK-9 activity is required for elongation as in higher metazoans (Fig. 2C). In contrast, germline Ser2-P may not require CDK-9 because (as in yeast) CDK-9 is not required for transcription elongation in the germline (Fig. 2B). While speculative, it is exciting to consider that transcription elongation may be differentially regulated in a tissue-specific manner in some metazoan systems.

**Perspective**

After an exciting 20 years of research on Pol II CTD phosphorylation and transcription, we are developing a clearer picture of the relationship between Ser2-P and Pol II elongation. As we have uncovered species-specific differences in Pol II regulation, it is also becoming clear that differences in Ser2-P can exist within a species as tissue-specific regulation. Thus, it is important that we begin to dissect mechanisms of Pol II elongation in distinct tissues.

Future tissue-specific studies are likely to be aided by new methods that isolate cell-type specific nuclei. Importantly many previous genome-wide studies of Pol II elongation regulation in metazoans have been performed using mixed cell-types. Based on the recent data suggesting tissue-specific regulation, it is possible that a mixture of cell types masks details of tissue-specific Pol II regulation. Isolation of tissue-specific material, either through flow cytometry, cell-type specific tagging, or the recently developed INTACT (isolation of nuclei tagged in specific cell types) method is likely to have a major impact on the ability to understand tissue-specific phenomenon. This method has already been used to isolate C. elegans muscle nuclei and it seems likely that this could be easily adapted to additional somatic tissues or the germline. As we move toward the development of methods that can dissect these events in multicellular organisms, it is likely that we will find unique exceptions to well-established models of transcription. Powerful genetic model systems such as C. elegans will continue to provide this unique insight.

**Disclosure of Potential Conflicts of Interest**

No potential conflict of interest was disclosed.

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