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The Paf1 Complex: a keystone of nuclear regulation operating at the interface of transcription and chromatin

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Abstract

The regulation of transcription by RNA polymerase II is closely intertwined with the regulation of chromatin structure. A host of proteins required for the disassembly, reassembly, and modification of nucleosomes interacts with Pol II to aid its movement and counteract its disruptive effects on chromatin. The highly conserved Polymerase Associated Factor 1 Complex, Paf1C, travels with Pol II and exerts control over transcription elongation and chromatin structure, while broadly impacting the transcriptome in both single cell and multicellular eukaryotes. Recent studies have yielded exciting new insights into the mechanisms by which Paf1C regulates transcription elongation, epigenetic modifications, and post-transcriptional steps in eukaryotic gene expression. Importantly, these functional studies are now supported by an extensive foundation of high-resolution structural information, providing intimate views of Paf1C and its integration into the larger Pol II elongation complex. As a global regulatory factor operating at the interface between chromatin and transcription, the impact of Paf1C is broad and its influence reverberates into other domains of nuclear regulation, including genome stability, telomere maintenance, and DNA replication.

Graphical Abstract

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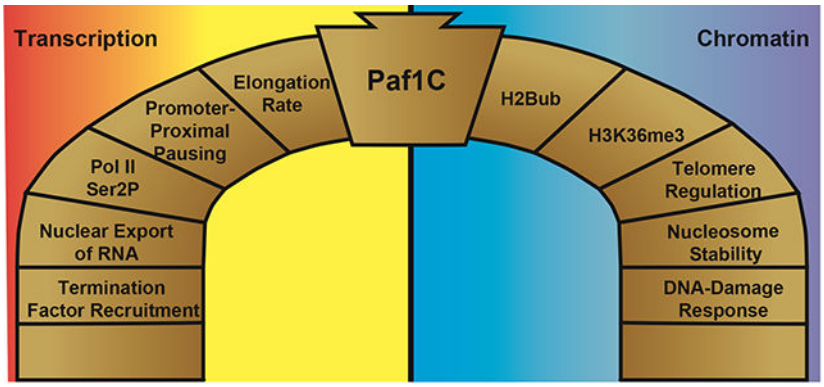

Keywords

Paf1C; RNA polymerase II; transcription elongation; histone modifications; post-transcriptional regulation

Introduction

In eukaryotes, all nuclear processes are profoundly influenced, directly or indirectly, by the packaging of the genome into chromatin. Comprised of an octamer of histones H2A, H2B, H3 and H4 and ~147 bp of DNA, nucleosomes represent the fundamental repeating unit of chromatin [1]. Access to DNA is strongly impacted by nucleosomes, emphasizing the importance of cellular mechanisms that regulate chromatin in a dynamic and controlled fashion. With respect to gene expression, all steps in the RNA polymerase II (Pol II) transcription cycle, from promoter recognition to transcript elongation and termination, are regulated by the positioning, occupancy, or modification states of nucleosomes [2]. Intriguingly, the transcription factors that regulate Pol II activity and the chromatin regulatory factors that alter nucleosome structure are engaged in an intricate interplay. Thus, transcription both controls and is controlled by chromatin structure. Protein factors that function at the interface of transcription and chromatin are, therefore, of fundamental importance to the interpretation and maintenance of the genome. The focus of this review is one such factor, the Polymerase Associated Factor 1 Complex, Paf1C.

Paf1C was first identified and characterized in the budding yeast *Saccharomyces cerevisiae* as a Pol II-interacting complex [3–5]. Through a convergence of biochemical and genetic studies, yeast Paf1C was shown to contain five subunits, Paf1, Ctr9, Cdc73, Rtf1, and Leo1, and function in the elongation stage of the Pol II transcription cycle [6–12]. Subsequent work revealed strong conservation of PAF1C subunits across eukaryotes, including humans, with some differences in the stability and composition of the minimally defined complex (detailed below) [13–15]. (A note on nomenclature: throughout the review, we will use lower case to designate yeast Paf1C or when we refer to the complex in general and uppercase to specify PAF1C in humans or other eukaryotes). Deletion of Paf1C subunit genes in yeast leads to a wide range of mutant phenotypes with evidence of subunit specificity [6,7,9,16–21]. These phenotypes include those that report on defects in transcription and chromatin structure, such as sensitivity to compounds that inhibit nucleotide biosynthesis, the

Suppressor of Ty (Spt^{-}) phenotype and cryptic transcription initiation [22–26]. Further, as a consequence of deregulated gene expression, yeast cells lacking certain Paf1C subunits exhibit defects in cell cycle progression and cell wall integrity [7,18,27]. Consistent with its pleiotropic effects, deletion of *PAF1* causes widespread changes in both the coding and noncoding transcriptome [28–30].

Although Paf1C is not essential for viability of yeast, mutation or depletion of PAF1C subunits in higher eukaryotes causes severe developmental defects, loss of stem cell pluripotency, altered immune responses to viral infections, and changes in neuronal migration that are associated with intellectual disability [31–41]. Moreover, numerous clinical manifestations have been attributed to overexpression or mutation of PAF1C subunit genes in humans. As examples, overexpression of *PAF1* has been associated with pancreatic and ovarian cancer, depletion of CTR9 alters the proliferative properties of certain breast cancer cells, and interactions between PAF1C and MLL promote leukemia [42–44]. An association between human cancers and CDC73, also known as the tumor suppressor protein parafibromin, predated knowledge that is was part of PAF1C. Heritable and somatic mutations in *CDC73* cause hyperparathyroidism jaw tumor syndrome and parathyroid carcinomas [45].

As in yeast, the diversity of phenotypes associated with deficiency or misexpression of PAF1C subunits in higher eukaryotes can be attributed to deregulation of gene expression and downstream processes. The goal of this article is to provide a comprehensive and current review of Paf1C. We present recent advances that have shed light on structure and mechanisms of transcription, chromatin regulation, and post-transcriptional events. Lastly, we describe connections of this highly conserved, multifunctional complex to nuclear processes beyond transcription (Figure 1).

Regulation of transcription elongation

Through its association with Pol II, Paf1C localizes to the bodies of actively transcribed genes. There, it engages in a network of functional and physical interactions with other members of the core Pol II elongation machinery. The mechanisms that direct Paf1C to Pol II appear to differ between species, as do the precise patterns of complex localization. In yeast, Paf1C is largely enriched on gene bodies with significantly less occupancy near promoters or in the termination region [10,30,46]. In contrast, PAF1C occupancy in mammalian cells can be detected throughout the transcription unit, including at promoters and downstream of the cleavage and polyadenylation site (CPS) [47]. Optimal occupancy of Paf1C on transcribed chromatin in yeast requires domains within Cdc73 and Rtf1 for which structural insight is available, as well as RNA binding by Leo1 (described in more detail below). In higher eukaryotes, the importance of RTF1 in PAF1C recruitment is unclear [14]. However, several studies performed in Drosophila and human cells demonstrated binding of developmentally important transcriptional activators to a region in CDC73 that is not conserved in the yeast protein, indicating that promoter-bound factors can recruit PAF1C to stimulate gene expression [35,48–50]. At genes regulated by the transactivator Myc, PAF1C is recruited through an interaction with Myc and then released to control productive elongation when Myc is proteolytically turned over [51]. While much remains to be learned

about the molecular mechanisms of Paf1C recruitment at specific genes in different systems, some common themes have emerged. For example, Paf1C association with Pol II in both yeast and human cells requires the activities of protein kinases, including CDK7 (Kin28 in *S. cerevisiae*) and CDK9 (Bur1 in *S. cerevisiae*), that phosphorylate the Pol II C-terminal domain (CTD) and/or the transcription elongation factor Spt5 (subunit of DSIF in humans) [52–55]. A complete understanding of how these regulatory factors, and potentially others such as noncoding and nascent transcripts, coordinate Paf1C recruitment will require further study. Importantly, independently of the exact mechanisms through which Paf1C is coupled to Pol II, there is strong support for the idea that, once recruited, it regulates the efficiency through which Pol II transits a gene (Figure 1).

Pol II Transcription

Early indications that Paf1C regulates transcription elongation by Pol II came from genetic and biochemical studies in yeast. When combined, mutations in genes encoding Paf1C subunits and proteins previously implicated in transcription elongation cause severe synthetic growth defects. These proteins include the well-studied transcription elongation factors TFIIS and Spt4-Spt5 and the histone chaperones FACT (complex containing Spt16 and Pob3) and Spt6 [6,9,56]. Further, loss of Paf1C subunits increases the cell's dependence on a fully functional Pol II CTD and the protein kinases and phosphatases that control CTD phosphorylation and elongation factor recruitment [6,9,21]. In addition to providing strong links between Paf1C and transcription elongation, these genetic studies strengthened the notion of subunit-specific functions within the complex, a recurring theme in this review. Typically, deletions of *PAF1* and *CTR9* confer the strongest single and double mutant phenotypes, consistent with their roles in maintaining structural integrity of the complex [13,57]. In parallel with the genetic studies, purification of Paf1C and chromatin immunoprecipitation experiments revealed physical interactions with proteins that regulate transcription elongation, such as FACT and Spt5, as well as enrichment of Paf1C on the bodies of active genes [6,8,10–12].

While these genetic and physical interactions are strongly supportive of a role for Paf1C in transcription elongation, subsequent studies provided more direct evidence that Paf1C stimulates transcription elongation. In assays designed to monitor Pol II progression through long, GC-rich templates *in vivo*, yeast cells lacking Paf1, Cdc73, Rtf1, and Leo1 exhibited decreased elongation efficiency [58–60]. More recently, depletion of PAF1 from a mouse myoblast cell line caused accumulation of Pol II on gene bodies, a slower Pol II elongation rate, and reduced Pol II processivity [47,61]. These cell-based assays complement *in vitro* transcription experiments, which incorporated purified proteins. Using a defined *in vitro* system with purified human factors and a chromatin template, Kim *et al.* demonstrated a direct stimulatory effect on transcription elongation of a six-subunit PAF1C, which included RTF1, a subunit that is less stably associated with PAF1C in metazoans [13,62]. This stimulatory effect of PAF1C on elongation was synergistic with that of TFIIS and independent of its roles in promoting histone modifications (described in more detail below). More recently, in reactions containing the elongation factor SPT6, purified PAF1C lacking RTF1 was shown to directly stimulate elongation in a minimal reaction lacking histones [55]. However, upon addition of RTF1, elongation was further stimulated [63]. Interestingly,

another study showed that RTF1 can stimulate elongation independently of its association with PAF1C [14]. These results argue for a direct stimulatory effect of multiple subunits within PAF1C on transcription elongation, including the dissociable RTF1 subunit.

Beyond a general effect on elongation efficiency, PAF1C is a key contributor to the regulation of promoter-proximal pausing of Pol II in metazoans, a widespread event in which Pol II pauses elongation ~20–50 bp downstream of the TSS [64]. Pausing of Pol II at this position, followed by its release into the gene body, is coordinated by several protein complexes. Negative elongation factor (NELF) and DRB sensitivity-inducing factor (DSIF, composed of SPT4 and SPT5) enforce pausing of Pol II, and pause release is triggered by the CDK9 kinase subunit of P-TEFb, which phosphorylates residues within NELF, DSIF, and the Pol II CTD. These phosphorylation events induce the release of NELF, the recruitment of elongation factors, and the transition of Pol II into productive elongation. The role of PAF1C in promoter-proximal pausing and release appears to be complex and potentially gene and cell-type specific. Two groups employing similar strategies of PAF1 depletion from cancer cell lines followed by whole-genome analysis of Pol II distribution arrived at opposing views. One group described a positive role for PAF1C in promoting Pol II phosphorylation and release into productive elongation [53]. Another group working with a different cell line concluded that PAF1C inhibits pause release by binding to and attenuating the functions of enhancers [65,66]. Of note, a very recent study reported occupancy of Paf1C at enhancers in mouse embryonic stem cells but concluded that Paf1C potentiates enhancer function [67]. More work is clearly needed to understand the differences among these observations. However, it is worth noting that support for both negative and positive effects of PAF1C on the transition to elongation has come from diverse studies. A genetic screen in zebrafish identified CDC73 and other PAF1C subunits as negative regulators of transcription elongation at genes important for blood development [68]. In contrast, the PHD-finger protein Phf5A has been shown to stabilize PAF1C occupancy on chromatin through a direct interaction and facilitate Pol II progression into elongation at genes important for embryonic stem cell maintenance [69]. Finally, data from recent structural studies favor a model in which PAF1C stimulates release of Pol II from promoter-proximal pause sites, as binding of PAF1C to Pol II requires P-TEFb and sterically occludes NELF from the elongation complex [55].

Pol I and Pol III Transcription

In addition to its effects on Pol II transcription, Paf1C has been implicated in regulating transcription by RNA polymerases I and III. Pol I is required for the synthesis of ribosomal RNAs (rRNAs), including 18S, 5.8S, and 25S rRNA, which are generated from a long precursor 35S rRNA. Transcription of rDNA accounts for the majority of transcription in log phase growth [70]. ChIP analysis demonstrated that Ctr9, Paf1, and Cdc73 occupy sites within the rDNA, suggesting that Paf1C travels with Pol I and regulates transcription elongation [71,72]. Because Pol I can bind directly to Spt5, Paf1C may be recruited to the rDNA through its interaction with Spt5 [63,73–77]. Measurement of nascent radiolabeled rRNA transcripts in yeast deletion mutants suggested a requirement for Paf1, Ctr9, and to a lesser extent Cdc73, in maintaining transcription efficiency on rDNA *in vivo,* and subsequent biochemical studies demonstrated a stimulatory effect of Paf1C on transcription

elongation by Pol I *in vitro* [71,78]. Consistent with a role in Pol I elongation, *paf1*Δ mutants exhibit defects in transcription-coupled rRNA processing [78], transcriptional readthrough at a Pol I reporter gene [72], and negative genetic interactions with deletion of *RPA49,* a gene encoding a nonessential Pol I subunit important for the control of transcription elongation [78]. Further insights into Paf1C regulation of Pol I transcription are discussed in other recent reviews [79,80].

More recently, Paf1C has been described as a negative regulator of tRNA expression by Pol III [81]. Upon deletion of *PAF1*, Pol III occupancy and expression of certain tRNA genes increases [81]. This role of Paf1 in restricting Pol III occupancy appears to be important for preventing DNA damage that stems from replication fork collapse near some tRNA genes [81]. The lack of correlation between Pol III and Paf1C occupancy suggests that Paf1C may not be directly recruited by Pol III, and there are gene-specific effects of Paf1C on Pol III transcription that remain to be elucidated [81]. Though much remains unknown about how Paf1C regulates transcription by Pol I and Pol III, it is evident that Paf1C broadly regulates the eukaryotic transcriptome.

Understanding the functions of Paf1C through structure

The structural basis of the many Paf1C functions, including how Paf1C subunits interact with one another and with other core members of the Pol II elongation complex, has remained elusive until recently. The individual Paf1C subunits interact in stoichiometric amounts [5,82], and in yeast, the five subunits-- Paf1, Ctr9, Cdc73, Leo1, and Rtf1-- are stably associated within the complex. In contrast, RTF1 appears not to be stably associated with PAF1C purified from human cells, despite the strong structural and functional conservation of Paf1C subunits [13,14]. However, it is worth emphasizing that some biochemical studies, including those that identified a direct stimulatory effect of PAF1C *in vitro*, were performed with PAF1C containing RTF1 [13,62]. In another distinction between the human and yeast complexes, human PAF1C includes the protein WDR61, a homologue of yeast Ski8, which is not present in the minimal yeast complex [15].

Structural analysis of protein domains

Initial efforts to elucidate Paf1C structure focused on individual domains, primarily those that tether it to the Pol II elongation complex (Figure 2). Three subunits, Cdc73, Rtf1, and Leo1, have been implicated in recruiting and maintaining Paf1C occupancy on transcribed chromatin [74,83–85]. These subunits can act independently in anchoring Paf1C to the Pol II elongation complex and do so through distinct mechanisms. Elimination of one tether point does not completely ablate Paf1C occupancy on chromatin, suggesting that several distinct interaction sites contribute to the proper coupling of Paf1C to Pol II [74,84].

Domains within Cdc73 and Rtf1 that engage in protein-protein interactions and tether Paf1C to the Pol II elongation complex have been identified. In contrast, Leo1 appears to mediate an interaction between Paf1C and elongating Pol II through its RNA binding activity, however the details remain unclear [84]. Crystallographic and molecular studies revealed that a conserved C-terminal region in Cdc73, termed the C-domain, adopts a Ras-like fold and is required for optimal occupancy of Paf1C on chromatin [83,86]. Based on *in vitro*

binding assays, a likely interaction partner for the Cdc73 C-domain is the phosphorylated Pol II CTD, although this has not been confirmed at the structural level [52]. With respect to *CDC73* mutations associated with hyperparathyroidism jaw tumor syndrome, many are predicted to truncate the protein or disrupt the hydrophobic core of the CDC73 N-terminal domain, which is more weakly conserved between yeast and humans than the C-domain [87–90] (Figure 2).

To secure Paf1C on chromatin, the Cdc73 C-domain functions in parallel with a highly conserved domain in Rtf1 termed the Plus3 domain (also termed the ORF Association Region, OAR) [74,75,83]. Initial studies in yeast showed that Rtf1, through its Plus3 domain, binds to the phosphorylated carboxy-terminal repeat region (CTR) of Spt5 [75]. The repeats of the Spt5 CTR are phosphorylated by Cdk9 (Bur1 in *S. cerevisiae*) and serve as an interaction scaffold for Paf1C and additional regulatory factors, analogously to the Pol II CTD [77,91]. The human RTF1 Plus3 domain structure has been solved by X-ray crystallography in complex with a phosphorylated SPT5 CTR peptide [74]. To accommodate binding to the phosphorylated SPT5 CTR, the Plus3 domain relies on two functionally important regions: a phospho-threonine recognition surface and a hydrophobic peptide-binding surface [74]. Separately, an NMR analysis of the RTF1 Plus3 domain highlighted structural conservation with nucleic acid binding proteins and revealed affinity of the Plus3 domain for ssDNA substrates that mimic the transcription bubble; however the functional role of this interaction in transcription requires further investigation [92]. Given its absence from purified PAF1C, the extent to which RTF1 remains associated with PAF1C and Pol II throughout the transcription cycle in human cells has not been fully explored. However, additional structural evidence discussed more fully below has recently emerged.

In addition to the human RTF1 Plus3 domain, the histone modification domain (HMD) of yeast Rtf1 has been solved by X-ray crystallography [46]. Required to stimulate ubiquitylation of H2BK123 in yeast (H2BK120 in humans; hereafter H2Bub), the Rtf1 HMD(74–139) is composed of a short loop followed by two alpha helices. Together, the alpha helices contain several conserved residues that are required for H2Bub and for direct interaction with Rad6, the ubiquitin conjugase for H2B [46]. The epigenetic context and consequences of HMD-stimulated H2Bub are described in greater detail in a subsequent section.

Structural analysis of subcomplexes

Moving beyond domain structures, crystallographic analyses of PAF1C subcomplexes have provided valuable insights into PAF1C assembly (Figure 2). Initial structural information on PAF1C subunit interactions came from crystallographic analysis of a fused heterodimer composed of internal regions of human PAF1 (161–250) and LEO1 (370–462) [57]. The structure revealed a PAF1-LEO1 interface stabilized by interactions between antiparallel β-sheets. Subsequently, crystal structures of human and *Myceliophthora thermophila* subcomplexes revealed details of the interactions between PAF1 and CTR9 [93,94]. N-terminal regions of CTR9 and PAF1 are required for heterodimer formation and the full assembly of the remaining complex members [93]. The N-terminal 120 amino acids of *M. thermophila* PAF1 adopt a hook-like structure with an extended 140Å region followed by

two shorter regions separated by ~90° bends [94]. Within a PAF1-CTR9-CDC73 ternary complex, 21 tetratricopeptide repeats (TPRs) in CTR9, comprising 42 consecutive α-helices, form right-handed superhelical turns that encircle the ~140Å length of the PAF1 N-terminal domain [94]. CDC73 contacts the PAF1-CTR9 subcomplex, wedging within one central groove between interior contacts of the PAF1-CTR9 twist. These subcomplex structures have yielded high-resolution views of the core interfaces involved in the assembly of PAF1C and revealed that certain cancer-associated missense mutations map to the PAF1-CTR9 interface [93,94].

Structural analysis of Paf1C in the context of the Pol II elongation complex

Insight into the architecture of the full PAF1C has been greatly advanced by the recent publication of multi-protein structures solved by cryo-electron microscopy (cryo-EM) [55,82]. These structures have also yielded contextual information about the transcription elongation machinery, including PAF1C occupancy on Pol II in conjunction with the elongation factors TFIIS, DSIF (SPT4-SPT5), and SPT6 [55,82]. An initial cryo-EM analysis of the five-membered *S. cerevisiae* Paf1C in complex with Pol II and TFIIS suggested that Paf1C forms a tripartite arrangement [82]. The high mobility of the subunits limited the resolution of this structure, though protein crosslinking combined with mass spectrometry clarified Paf1C organization. Ctr9, which composes the central lobe of Paf1C in this structure, crosslinks to a Paf1-Leo1 heterodimer that forms a second lobe in the tripartite arrangement. Cdc73 projects laterally from the central Ctr9 subunit to form the third lobe. The positioning of Rtf1 was not observed in this model, though crosslinking data showed that the C-terminus of Rtf1 is anchored to Paf1C via the Paf1-Leo1-Ctr9 subcomplex in agreement with previous mutational studies [95]. As observed by pulldown assays, TFIIS stimulates Paf1C binding to Pol II [82]. This observation is in line with previous investigations showing that PAF1C and TFIIS act synergistically to facilitate transcription elongation *in vitro* [13]. Synergism of TFIIS and PAF1C during transcription elongation is dependent on PAF1 and LEO1 directly binding TFIIS in the presence of Pol II [13].

The cryo-EM structure of an activated elongation complex, which included *Sus scrofa* Pol II and human PAF1C (lacking RTF1), SPT6, and DSIF, provided higher resolution imaging for the positions of PAF1C subunits [55]. As expected from the crystallographic studies on subcomplexes containing PAF1 and CTR9 [93,94], the cryo-EM structure revealed that CTR9 is largely composed of TPR motifs that wrap PAF1. The TPR motifs are followed by a vertex domain that transitions into a trestle domain, a long extension that branches 100Å distally toward the Pol II foot. The vertex and adjacent TPR motifs are major contact points for WDR61, which forms a seven-bladed ß propeller structure [96]. The CTR9 trestle also forms contacts with Pol II at RPB5 near downstream DNA. Furthermore, an N-terminal LEO1 extension is visible in this structure and extends proximally to upstream DNA, putatively stabilizing the single-stranded DNA in the transcription bubble. With the exception of a limited contact with a CTR9 TPR motif, CDC73 appears to be a flexible protein with minimal observable density in this cryo-EM structure. While RTF1 was not included in this initial structure of an activated elongation complex, its position within the Pol II elongation complex has been recently elucidated [55,63], discussed more fully below.

Importantly, the structure of the activated elongation complex provides an avenue to interpret the role of PAF1C within the context of promoter-proximal pausing and release. Comparison of the paused elongation complex [97] to the active complex demonstrates that NELF positioning at the foot of Pol II is sterically incompatible with PAF1C binding because CTR9 and WDR61 bind NELF-occupied territory. These data provide evidence for PAF1C occupancy in the activated elongation complex and exclusion from the paused elongation complex. Therefore, these structural data support a role for PAF1C in pause-release.

Building on their analysis of the activated Pol II elongation complex, the Cramer lab recently reported the cryo-EM structure of a complex containing RTF1 in addition to Pol II-DSIF-PAF-SPT6 [63] (Figure 3). This structure revealed unexpected interactions between RTF1 and Pol II as well as a role for RTF1 in stimulating transcription elongation [63]. In this activated complex, RTF1 stimulates the transcription elongation rate of Pol II five-fold in a manner that is dependent on DSIF and PAF. The available structure of the RTF1 Plus3 domain in complex with the SPT5 CTR helped clarify the positions of other structural elements within RTF1 [74]. The positions of several RTF1 helices C-terminal to the Plus3 domain were identified. One such helix, the RTF1 fastener domain is positioned within a groove on the Pol II surface, likely functioning to secure PAF1 to Pol II. Importantly, the C-terminal ‘latch’ is a loop domain which approaches the bridge helix within the active site of Pol II. RNA extension assays demonstrated that the latch loop of RTF1 is required for the stimulatory effect of RTF1 on transcription elongation *in vitro*, highlighting a novel and likely significant role for the mammalian RTF1 protein [63].

With the increasing resolution of PAF1C within the active elongation complex, the overall architecture of PAF1C has been modified from the initial tripartite structure to a more concise dual part structure [63,82] (Figure 3). One branch, including WDR61, CTR9, CDC73, and the N-terminus of PAF1 is linked to a PAF1-LEO1 branch. The dual structure is connected by a PAF1 extension, and RTF1 stabilizes these two branches via interactions with both halves of the complex. These new structural insights into RTF1 provide evidence for its roles in maintaining PAF1C occupancy and stimulating elongation rate by allosteric interaction with the bridge helix of Pol II. Despite these advances, the mechanisms that control the association of RTF1 with PAF1C in humans remain to be determined.

Paf1C control of transcription-coupled processes

The impact of Paf1C on chromatin-templated processes extends beyond direct control of the transcription cycle. Eukaryotic cells deploy an arsenal of resources to establish and maintain chromatin structure. Electrostatic and steric heterogeneity in chromatin structure and modification state can recruit, repel, and modulate the activities of factors on chromatin. These processes are essential for regulating the landscape of chromatin-templated events [98]. To maintain such multitudinous markings, cells must carefully coordinate the deposition, erasure, and reading of post-translational modifications of histones. A rich history of evidence connects Paf1C directly and indirectly to chromatin maintenance through several pathways (Figure 1).

H2Bub, H3K4me, H3K79me

Histone post-translational modifications (PTMs) are a major mechanism through which Paf1C shapes the chromatin template and therefore regulation of transcription. Early studies established the importance of Paf1C for the conserved histone PTM, H2B mono-ubiquitylation [99,100]. H2BK123 (H2BK120 in humans) is mono-ubiquitylated by the ubiquitin-conjugating enzyme Rad6 (RAD6A and RAD6B in humans) and the ubiquitin-protein ligase Bre1 (RNF20 and RNF40 in humans) in a co-transcriptional process that is promoted by the co-factor Lge1 [62,101–104].

As part of the elongation complex traveling with Pol II, Paf1C couples H2Bub deposition to active transcription through the recruitment and stimulation of Rad6 and Bre1. Rtf1, Paf1, and Ctr9 have all been reported as necessary for H2Bub and for the PTMs that require H2Bub as a prerequisite, namely di- and tri-methylation of H3K4 and H3K79; however the role of Rtf1 in regulating H2Bub is best understood [46,62,99–101,105–108]. Genetic and biochemical studies identified a small domain within Rtf1, the histone modification domain (HMD), as both necessary and sufficient for stimulating deposition of H2Bub *in vivo* [46,95,106]. Expression of the Rtf1 HMD alone restores H2Bub levels in *S. cerevisiae* strains deleted for the *RTF1* gene or all five Paf1C-encoding genes [46]. However, the mis-localization of H2Bub in these strains argues that other domains within Rtf1, such as the Plus3 domain, and other Paf1C subunits, such as Cdc73 and Leo1, are important for targeting the HMD and hence H2Bub to active genes. The HMD directly contacts Rad6 *in vivo* and a recombinant HMD protein can stimulate H2Bub *in vitro* in a reaction reconstituted with recombinant factors and lacking transcription, suggesting that the HMD constitutes a cofactor domain that directly participates in the H2Bub reaction [46]. Consistent with this idea, the HMD also makes direct contact with the nucleosome acidic patch, as measured through *in vivo* site-specific crosslinking experiments, and amino acid substitutions to the acidic patch reduce H2Bub levels in yeast cells [109–111].

The occupancy of Paf1C, and thus Rtf1, on chromatin is highest at sites of most active transcription [10,46]. A requirement for Paf1C in H2Bub deposition is therefore concordant with other observations showing that levels of H2Bub on chromatin are proportional to the transcriptional activity over a gene [10,101,112,113]. Furthermore, H2Bub is a highly dynamic modification. Deubiquitylases, Ubp8 (USP22 in humans) and Ubp10 (USP36 in humans) rapidly act to erase H2Bub [114–119]. Thus, any basal H2Bub deposition that might occur in the absence of Rtf1 is undetectable *in vivo*.

Interestingly, recent biochemical observations have provided evidence that H2Bub deposition occurs in liquid-liquid phase-separated condensates [120]. Such condensates are formed through interactions with the intrinsically disordered domain of Lge1, which is followed by a C-terminal coiled-coil domain necessary for the recruitment of Bre1. Although H2Bub deposition has been argued to involve the recruitment of Rad6 and nucleosomes to the condensate, the question of how Paf1C fits into this model remains unexplored. To fully characterize how Rtf1 stimulates H2Bub in live cells, the role of the HMD in recruitment and stimulation of Rad6-Bre1 will need to be reconciled with the Bre1-Lge1 condensate model.

H2Bub participates in a variety of physiological processes [121–123]. At the molecular level, some *in vitro* observations suggest H2Bub-containing nucleosomes are modestly destabilized and discourage higher-order compaction [124,125]. Other studies suggest that nucleosome stability is promoted by the presence of H2Bub [126]. Yeast nucleosomes enriched for H3K123ub show lower rates of turnover and *ubp8Δ* strains exhibit increased nucleosome occupancy [127,128]. Furthermore, H2Bub-containing nucleosomes provide a heightened energetic barrier for elongating Pol II relative to unmodified nucleosomes [129]. H2Bub has been observed to promote occupancy of FACT, an essential histone chaperone, on the *GAL1* ORF, and current models suggest that FACT and Ubp10 use the ubiquitylation state of H2B to coordinate the reassembly of nucleosomes in the wake of transcription and replication-induced destabilization [130,131]. Reciprocally to FACT dependence on H2Bub for occupancy, observations in both yeast and metazoan systems suggest that FACT, in turn, stimulates H2Bub deposition [130,132]. Though globally decreased upon DNA damage, H2Bub has also been observed as selectively enriched at DNA damage-regulated genes and, in conjunction with FACT, increases chromatin exchange dynamics in response to DNA damage [133,134].

H2Bub has long been known to cross-talk with other histone PTMs [135]. In humans, PAF1C promotes H2BK34ub and H4K16ac through direct interactions with the MOF-MSL complex [136]. However, there is evidence for codependence between H2BK34 and H2BK120 ubiquitylation, and independent from H2BK120ub, H2BK34 promotes H3K4me3 and H3K79me2 in metazoans [136,137]. In both yeast and humans, H2Bub is required for di- and trimethylation of H3K4 and H3K79 [138–143]. The Set1 and Dot1 methyltransferases catalyze H3K4me and H3K79me, respectively. A large number of recent cryo-EM structures have elegantly revealed extensive interactions between ubiquitylated nucleosomes and the methyltransferases, providing new insights into H2Bub-stimulated H3 methylation by Dot1 and Set1 [144–149]. H2Bub additionally stimulates Rad6/Bre1-mediated ubiquitylation of COMPASS subunit, Swd2, which itself independently promotes H3K4me2/3 at the 5’-ends of genes [150]. The role of H2Bub in promoting histone methylation makes it unsurprising that mutants in Paf1C subunits also reduce levels of H3K4me2/3 and H3K79me2/3 [20,101,105,107]. However, through a mechanism that may be independent of Rad6, members of Paf1C (Paf1 and Rtf1) promote full recruitment of Set1 [105]. Moreover, Paf1 and Leo1 have been reported to bind histone H3 *in vitro,* providing a mechanism for direct interactions between Paf1C and nucleosomes [57]. These findings provide hints that Paf1C may be playing a role in the activation of methyltransferases distinct from H2Bub.

H3K36me3

All forms of H3K36me in yeast are mediated by Set2 [151]. H3K36me3 is enriched toward the 3’ ends of active genes and promotes deacetylation of H3 and H4 through activation of the Rpds3 histone deacetylase complex [152–157]. The deposition of H3K36me3 is critical to reset chromatin in the wake of nucleosome transactions and suppress spurious initiation events at cryptic promoters within open reading frames (ORFs) [98,158]. Yeast strains deleted for *PAF1* and *CTR9* are strongly deficient in H3K36me3 and show evidence for defective suppression of cryptic transcription [21]. While the loss of *CDC73* mildly affects

H3K36me3 levels, deletion of *RTF1* or *LEO1* does not seem to impact this mark, further illustrating the functional diversity among Paf1C subunits [21].

In addition to its roles in histone PTMs, Paf1C is important for phosphorylation of the Pol II CTD at serine 2 (Ser2P) in yeast, likely acting by activating Ctk1, the major Ser2 kinase, or antagonizing the action of its cognate phosphatase, Fcp1 [159–161]. Consistent with a conserved function in promoting CTD Ser2P, knockdown of PAF1 in THP1 acute myeloid leukemia cells decreased genic occupancy of the human homologue of Ctk1, CDK12 [53]. Set2 is recruited to Pol II through its Set2-Rpb1 interacting (SRI) domain, which recognizes Ser2P [162]. The interaction between the Set2 SRI-domain and the Ser2P-modified CTD is needed to promote full levels of H3K36me2/3 in *S. cerevisiae* and H3K36me3 in *Schizosaccharomyces pombe* [152,162,163]. Therefore, the loss of Ser2P observed in Paf1C mutants likely reduces Set2-mediated deposition of H3K36me on chromatin [164].

Nucleosome Occupancy

Given the changes in histone modification levels observed in cells lacking or mutated in Paf1C, it is not surprising that nucleosome occupancy is also impacted in these cells. However, the limited available data on nucleosome occupancy in the absence of Paf1 paint a complex picture. In agreement with an earlier study focusing on a highly expressed locus, one MNase ChIP-seq analysis of an *S. cerevisiae paf1Δ* strain revealed a slight decrease in genic nucleosome occupancy relative to wild type when normalized to total nucleosome content in bulk chromatin [128,165]. This study reported a similar reduction in nucleosome occupancy in an H2BK123A mutant, which lacks the site of H2B ubiquitylation [128]. Therefore, a reduction in Rtf1-mediated ubiquitylation of H2BK123 likely contributes to the lower nucleosome occupancy levels in the *paf1Δ* strain, where Rtf1 levels are low [85]. In contrast, upon siRNA-mediated knockdown of PAF1 and CDC73 in C2C12-derived myotubule cultures for 48 h, nucleosome occupancy was unchanged as determined by H3 ChIP-seq [47]. Finally, and most recently, ATAC-seq and MNase-seq experiments in C2C12 myoblast lines depleted of Paf1 through both siRNA and doxycycline-induced repression for 2–4 days led to increased nucleosome occupancy [61]. This effect has been hypothesized to be caused by increased pausing of Pol II, thus reducing the disruption of genic chromatin. The discrepancies between these findings in yeast and mammalian systems may prove to be consequences of long-term indirect effects of null alleles in contrast to shorter-term depletion of Paf1C or a reflection of differences in how Paf1C coordinates the intersection of transcription and chromatin structure in yeast and mammals.

Paf1C has also been shown to interact with chromatin remodeling factors Chd1 and the Ino80 complex (Ino80C) [95,166,167]. A yeast-two-hybrid screen initially uncovered an interaction between Rtf1 and Chd1, linking the function of this chromatin remodeling factor to Paf1C and transcribed chromatin [167]. This interaction requires regions near the N-terminus of Rtf1 and C-terminus of Chd1 [95,167]. Several studies have reported a key role for Paf1C in recruiting Chd1 to active genes, though the studies differ in their conclusions about the importance of individual Paf1C subunits in this process. [95,167,168]. Additionally, Paf1C coimmunoprecipitates with Ino80C in yeast. As identified in mass spectrometry experiments, Cdc73 appears to be at least one anchor point between Ino80C

and Paf1C. Interestingly, current evidence supports a functional role for this interaction in dislodging Pol II from chromatin in response to replication stress [166].

Transcript termination, processing, and export

Paf1C disengages from Pol II on protein-coding genes at a location that coincides with the canonical CPS in yeast; however, mammalian cell lines show a lingering association with Pol II past the CPS [10,47]. Given these observations, it is perhaps expected that Paf1C is additionally implicated in transcription termination, 3'-end processing of transcripts, and co-transcriptional coupling of transcripts with nuclear export factors. Building on earlier studies, recent discoveries have strengthened the body of evidence relating Paf1C activities to the end of the transcription cycle.

Early studies in yeast revealed effects of Paf1C on the termination and 3'-end processing of both coding and noncoding Pol II transcripts. *S. cerevisiae* strains deleted for either *PAF1* or *RTF1* synthesize mRNAs with shortened polyA tails and have reduced levels of the 3' processing factor Pcf11 on protein-coding genes [85]. Additionally, in *rtf1Δ* and *cdc73Δ* strains, the association of Cft1 (homologue of human CPSF160) with Ser5P-modified Pol II is decreased ~10-fold [164]. While disruption of the coupling of Paf1C to Pol II via loss of Rtf1 or Cdc73 severely compromises the recruitment of RNA processing factors to transcribing Pol II, selective effects of Paf1 and Ctr9 on 3'-end processing have been described. For at least a subset of genes with alternative polyA sites, the absence of Paf1 in yeast leads to preferential use of the distal polyA site and 3'-extended transcripts [28]. Moreover, *paf1Δ* yeast cells produce 3'-extended transcripts from noncoding RNA loci, particularly snoRNA genes [29,169,170]. Termination of transcripts originating at these loci is dependent on the Nrd1-Nab3-Sen1 pathway and subsequent RNA processing by the TRAMP and RNA exosome complexes [171–176].

Paf1C likely regulates transcription termination and RNA 3'-end processing through several interrelated mechanisms, including but not limited to physical interactions with RNA processing factors [164,177]. For example, In the absence of Paf1C, the reduced elongation rate of Pol II may alter the kinetic coupling between transcription elongation and termination [178,179]. Furthermore, it has long been known that Ctk1, the *S. cerevisiae* Pol II CTD kinase that modifies Ser2, is required for proper recruitment of proteins associated with cleavage and polyadenylation to the 3' ends of genes, including Pcf11, Cft1, Cft2, Rna14, and Rna15 [180,181]. Additionally, the CDK12 kinase in human cells has been shown to be necessary for full recruitment of RNA cleavage factors, CSTF64, CPSF73, and CSTF77 [182,183]. These findings support the idea that, among other possibilities, reduced levels of Pol II Ser2P contribute to the mis-regulation of transcription termination and RNA 3'-end processing in Paf1C mutants.

Paf1C also appears to impact transcription termination and RNA 3'-end processing through its effects on chromatin structure. With respect to the NNS-dependent snoRNA genes, defects in several Paf1C-mediated histone modifications, including H3K4me3, H2Bub, and H3K36me3, lead to extended snoRNA transcripts possibly by influencing transcription elongation rate [20,170,184]. Interestingly, very recent observations suggest that the effects of Paf1C-dependent histone PTMs are not limited to snoRNA genes, as yeast cells lacking

Set1 or Set2 exhibit changes in alternative polyadenylation of mRNAs and elevated levels of Pol II Ser2P and 3'-end processing factors near CPS elements [185]. Moreover, given the Chd1-dependent suppression of H3 turnover at the 3' ends of long yeast genes, altered Chd1 occupancy patterns in Paf1C mutants may influence the chromatin state at the 3' ends of transcription units and consequently transcription termination [186]. Indeed, early studies in *S. pombe* uncovered a requirement for Chd1 in transcription termination [187]. Finally, direct or indirect contacts between the transcription termination machinery and pre-mRNA transcripts could be mediated by Paf1C. Consistent with this possibility, in *S. cerevisiae* all Paf1C subunits have been reported to bind to nascent pre-mRNA transcripts through PAR-CLIP experiments [188,189].

Ties between Paf1C and RNA 3'-end processing appear to be, in part, conserved in metazoans. Human CDC73 co-immunoprecipitates with 3' pre-mRNA processing factors, such as Symplekin and subunits of the CPSF and CstF complexes, in HEK 293T cells [177]. Concordant with these findings, mass spectrometry studies in C2C12 mouse myoblast cells have confirmed interactions between CDC73 and several termination and RNA cleavage factors including CPSF3, PCF11, and PPPR1R10 [61]. These biochemical interactions are supported by functional studies. Depletion of PAF1C subunits reduced the efficiency of transcript cleavage and polyadenylation both in cells and in an *in vitro* transcription-coupled cleavage and polyadenylation reaction [190]. Furthermore, mapping of polyadenylation sites using 3'READS upon depletion of PAF1, CDC73, and SKI8 in C2C12 myoblast cells demonstrated generally shortened 3'UTRs, as opposed to the extended transcripts observed at specific genes in *S. cerevisiae* [28,47]. In the same study, increased utilization of cryptic polyadenylation sites and upstream antisense RNA synthesis was observed in cells depleted for PAF1 and CDC73.

Paf1C also influences transcript fate post-termination. Paf1C functionally and physically associates with proteins implicated in nuclear mRNA export. Hpr1, a component of the THO/TREX RNA-export complex, copurifies with Cdc73 in yeast and was formerly thought to be a subunit of Paf1C [17,191]. Phenotypically, *S. cerevisiae* mutants lacking TREX components and certain Paf1C subunits exhibit common mutant phenotypes and show similar patterns of splicing mis-regulation suggesting a shared role in transcript regulation [17,192]. Consistent with a function of Paf1C in adapting transcriptional machinery to transcript export, in HEK 293T cells, CDC73 coimmunoprecipitates with the RANBP2 component of the nuclear pore complex and siRNA depletion of PAF1 leads to the nuclear accumulation of an mRNA [89,190]. Additionally, knockdown of CDC73 leads to increased accumulation and nuclear retention of histone transcripts in HCT116 and HeLa cells, respectively [193]. More recent studies in yeast extended the connections between Paf1 and RNA export. In one study by Mellor and colleagues, an adaptation of native elongating transcript sequencing (NET-seq) was used to map the location and measure the enrichment of Paf1-containing transcription elongation complexes genome-wide [30]. The authors demonstrated that a basal level of Paf1C occupancy on genes is necessary for controlling Pol II elongation, but higher levels of Paf1C association promote nuclear export over nuclear retention and degradation of transcripts [30]. Paf1C could further indirectly influence transcript fate through H2Bub, which is important for the subsequent ubiquitylation of Swd2, a component of both the yeast Set1-containing COMPASS complex and the APT

RNA 3'-end processing complex [194–196]. While Swd2 ubiquitylation occurs proximal to the 5'-end of a gene body, it is required for the full recruitment of the Mex67 transcript export receptor to RNA [197]. Finally, Bre1 has been reported to promote H2Bub and Pol II pausing immediately downstream of the CPS, though deletion of Paf1 and Rtf1 causes only minor changes to 3'-occupancy of Pol II [198]. Together, these studies demonstrate close ties between Paf1C and post-transcriptional events.

Ripples beyond transcription

Although Paf1C recruitment to chromatin depends largely, if not entirely, on its association with actively transcribing RNA polymerase, its influences on the epigenome and transcriptome reverberate into other domains of nuclear regulation. The physiological consequences of Paf1C deficiency or mutation are the culmination and integration of several interrelated events, including disruptions in Pol II elongation, Pol II termination, transcript processing, nucleosome post-translational modification, and nucleosome stability (Figure 1). Not only do these processes cross-talk between each other, they can also contribute to seemingly disparate, downstream biological processes (Figure 1). Excitingly, recent studies have uncovered mechanisms describing how dysfunctions in gene silencing, DNA repair, recombination, and replication can be elicited from mis-regulation of Paf1C-dependent processes.

Heterochromatin and telomere regulation

There are few domains of chromatin regulation independent of Paf1C influence. Even transcriptionally silent heterochromatin is disrupted in the absence of Paf1C. Early studies in budding yeast found that Rtf1, through its downstream histone modification functions, is required for telomeric silencing and full occupancy of the silencing factor Sir2 near telomeres [99,107]. Furthermore, in *S. pombe*, Leo1 appears to be a key player, functioning at the intersection of Paf1C and heterochromatin despite minor effects on H2Bub or H3K36me regulation relative to other Paf1C members [21,101,199]. In fission yeast lacking Paf1, Tpr1 (homologue of Ctr9), Leo1, and to a lesser extent Prf1 (homologue of Rtf1), H3K9me2, a heterochromatin-associated modification, spreads across heterochromatin boundaries [199,200]. This effect can be suppressed by artificially tethering Mst1, an H4 acetyltransferase, to chromatin of *leo1Δ* cells and does not depend on boundary-specific sequences [200]. The mechanism through which Leo1 and other members of Paf1C influence heterochromatin may be indirect, as Mst1 was not observed to co-immunoprecipitate with Paf1C subunits. Indeed, although Leo1 is conserved between budding yeast and fission yeast, H3K9 methylation is not. This suggests that Leo1 may have functionally diverged between the two yeasts, or more likely, the effect of Paf1C on heterochromatin is downstream of the primary activities of the complex. In agreement with the latter hypothesis, another study found that infiltration of H3K9me2 beyond heterochromatin boundaries coincides with decreased histone turnover [199]. Mis-localized H3K9me2 and reduced histone turnover were further observed in quiescent *leo1Δ* fission yeast, likely contributing to an inability to survive long periods of nitrogen starvation [201]. It is possible that the direct binding of nucleosomes by Paf1/Leo1 or Rtf1-mediated Chd1

recruitment may also play a role in reducing histone turnover in euchromatin or facultatively heterochromatic regions [202].

Regulation of transcription and chromatin by Paf1C impacts telomere regulation as well. An early screen of factors affecting telomere length revealed Rtf1 and Cdc73 as necessary for full-length telomeres [203]. Additionally, the levels of *TLC1*, the RNA component of the ribonucleoprotein telomerase, are reduced approximately two to four-fold in *S. cerevisiae* strains lacking individual Paf1C subunits, and overexpression of *TLC1* can partially suppress slow growth phenotypes of Paf1C-deficient strains [204]. Concordantly, cells lacking Paf1, Ctr9, and Cdc73 exhibited the greatest reductions in telomere length [204,205]. In contrast to the reduction in *TLC1* levels, the yeast telomere repeat-containing RNA, TERRA, accumulates in *paf1*Δ and *ctr9*Δ strains but not in *cdc73*Δ strains [206]. Interestingly, shortened telomeres have been reported to promote TERRA, due to decreased Rat1 exonuclease activity, and telomeric R-loops, due to reduced occupancy of Rif2 which recruits the R-loop-resolving RNase H2 [207,208]. This, in turn, prevents the resolution of R-loops and promotes a DNA damage response, followed by homology-directed repair of the telomeres, ultimately lengthening shortened telomeres and delaying the onset of senescence [208,209].

Genome stability, double-strand breaks, and meiosis

In the absence of Paf1C, double-strand break (DSB) repair, unprogrammed gross chromosomal rearrangements (GCRs), and programmed recombination events in meiosis are misregulated through multiple mechanistically distinct pathways. Yeast strains lacking Paf1, Ctr9, and Cdc73 show an increased frequency of GCRs [205]. In particular, coupling the *cdc73Δ* allele with other telomere shortening mutations (*tel1Δ* and *yuk1Δ*) synergistically reduces telomere length and stimulates GCRs, emphasizing the functional relationship between telomere maintenance and genome stability [204,205]. Intriguingly, a central domain of Cdc73 (residues 125–229 in yeast) is both necessary and sufficient to promote association with other Paf1C subunits, localize to the nucleus, and suppress GCRs [205].

Experiments with yeast artificial chromosomes additionally revealed that *cdc73Δ* and *leo1Δ* mutants exhibit an elevated frequency of GCR [210]. This phenotype can be suppressed by overexpression of RNase H1, a nuclease acting on DNA-RNA hybrids, suggesting that R-loops induce genome instability in the absence of functional Paf1C [210]. RNA-DNA hybrids between a transcript and the template strand are a normal part of cellular physiology; however, these R-loop sites can be cleaved as targets of the nuclear-excision repair endonuclease ERCC4 (also referred to as XPF), thus contributing to genomic instability [211].

Indeed, R-loop formation appears to factor into the relationship between Paf1C and genome stability in higher eukaryotes as well. One study found that the BRCA2 tumor suppressor gene promotes occupancy of PAF1C on chromatin which, in turn, is necessary to reduce the formation of 5’ R-loops near the site of promoter-proximal pausing [212]. Furthermore, R-loop accumulation in a BRCA2-mutant fibroblast cell line can be suppressed by overexpression of PAF1 [212]. Such findings are consistent with a model wherein PAF1-mediated resolution of promoter-proximal pausing prevents the formation of unscheduled R-

loops. Though Paf1C appears to negatively regulate R-loop formation, occupancy of Paf1C is enriched on genes that produce R-loops with particularly high enrichment over the promoters and termini of these loci [213]. The mechanism through which Paf1C influences R-loop formation requires more investigation; however, the deep ties between Paf1C and Pol II elongation kinetics or termination factor interactions could play a role in these phenomena.

Intriguingly, *paf1Δ*, *rtf1Δ*, *cdc73Δ*, and *leo1Δ* mutants in budding yeast exhibit a synthetic sensitivity to UV radiation in a *rad7Δ* background exclusively reliant on transcription-coupled nucleotide excision repair (TC-NER) [59]. Indeed, the relationship between Paf1C and DNA repair runs deep. The Rad26-dependent TC-NER pathway in yeast is stimulated by Paf1C; however, Rad26-independent TC-NER is inhibited by Paf1C in cooperation with Spt4-Spt5 [108]. These observations have led to a model in which stabilization of Pol II around a lesion by Paf1C and Spt4-Spt5 prevents efficient repair in the absence of Rad26 [108].

Outside its direct influence on transcription dynamics, Paf1C likely exerts control over the regulation of DSB repair and meiotic recombination through histone PTMs. Sites of high meiotic recombination frequency, so-called hotspots, are enriched at promoter regions; however, transcriptional frequency and low nucleosome occupancy are not sufficient to drive recombination [214–216]. Sites enriched for H3K4me3 show increased recombination rates while H3K36me3 and H3K79me3 are preferentially located at recombination cold spots [215]. Additionally, Paf1C mutants exhibit global genomic repair defects resulting from H3K79me2/3 loss [108]. H3K79me acts as a foothold for the Tudor domain of DNA-checkpoint protein Rad9 and is required for full activation of Rad53 in response to DNA damage [217–219]. Furthermore, H3K36me3 has been tied to DNA repair through its roles in recruiting homologous recombination (HR) repair machinery in human cells lines and shifting repair preference towards non-homologous end joining in yeast [220–222]. Serrano-Quílez *et al.* provide a detailed, contemporary discussion on the extensive and intimate relationship between H2Bub, H3K4me3, and meiosis [123].

DNA Replication

Paf1C assists in the navigation of collisions between the replisome and Pol II. When the transcription elongation complex meets a DNA replication fork, RNA synthesis needs to pause until the transcribed locus is replicated. Mutations in *LEO1*, *RTF1*, and *CDC73* were identified in a targeted screen in *S. cerevisiae* for synthetic interactors with the replisome-destabilizing *mec1-100* kinase allele under HU-induced replication stress conditions [166,223]. Indeed Leo1 was identified as a Mec1 target in a phosphoproteomic study [224]. This finding was followed by demonstrations of Ctr9, Paf1, and Rtf1 phosphorylation through *in vitro* kinase assays [166]. Both Mec1 and Paf1C are required to promote the eviction and degradation of Pol II colliding with the replisome. Fascinatingly, these studies indicate that while Paf1C positively regulates Pol II elongation, in the context of replication stress, Paf1C may assist in the removal of arrested Poll II. Much remains to be elucidated about this function of Paf1C and how PTMs of Paf1C subunits regulate their functions.

Additionally, the role of Paf1C in H3K4 methylation ties its function to the regulation of gene dosage homeostasis throughout the replication cycle. Cells are presented with a challenge of gene dosage regulation as early firing origins of replication duplicate some genes while others remain temporarily unduplicated. Unless gene expression of the duplicated genes is suppressed, the resultant imbalance in the gene expression program can be deleterious to the cell, as evidenced in conditions of unbuffered aneuploidy [225]. To solve this problem during replication, the deposition of transcriptionally activating H3K4me3 on transcribed chromatin is preferentially substituted with repressive H3K4me2 [226]. Naïve H3K56ac-marked histones that have been incorporated into newly replicated chromatin promote histone turnover and dilute H3K4me3 in favor of H3K4me2 which reduces gene expression until the trimethylation state is restored over time in a manner dependent on Paf1C and downstream of H3K56ac [226]. Mutants in Paf1C required for H2Bub deposition (Rtf1, Paf1, and Ctr9) and therefore H3K4me showed the strongest imbalance in dosage homeostasis with Leo1 and Cdc73 appearing dispensable for this function [226]. These findings emphasize that, as scientific knowledge of Paf1C expands, it becomes ever clearer that this transcription elongation complex modulates a broad range of chromatin-templated processes through a combination of direct and indirect mechanisms.

Concluding remarks

Decades of study in a variety of model systems have revealed Paf1C to be deeply tied to a complex web of connected regulatory mechanisms; yet, there is much more to be learned. While the subunits in Paf1C comprise a stable complex, it has become increasingly clear that these subunits exhibit a degree of functional specialization. As examples, stimulation of H2Bub by Paf1C can be tied directly to Rtf1, and histone binding has been attributed to the Paf1-Leo1 module. Future genetic and biochemical studies dissecting subunit-specific roles of Paf1C will further enrich understanding of precisely how individual subunits contribute to the whole of Paf1C function. New insights into Paf1C may also explain unresolved questions, including how Rtf1 participates in the Rad6/Bre1/Lge1 phase-separated catalytic condensate, what is the purpose of Paf1C binding to RNA, how are PAF1C and RTF1 recruitment regulated in metazoans, does Paf1C play a role regulating COMPASS and Dot1 independently of H2Bub, and how does Paf1C promote Pol II Ser2P. As innovative techniques to investigate chromatin-templated processes advance, they will accelerate the pace of discovery, thus paving the way for novel insights on this fascinating keystone of epigenetic regulation.

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Highlights:

- Paf1C subunits have distinct and shared roles in regulating transcription elongation and chromatin structure
- Paf1C functionally and physically interacts with the Pol II elongation complex and proteins required for histone modification and nucleosome remodeling
- Paf1C regulates gene expression at post-transcriptional levels through effects on transcript termination, processing and export
- Paf1C influences nuclear processes beyond transcription and epigenetic modifications

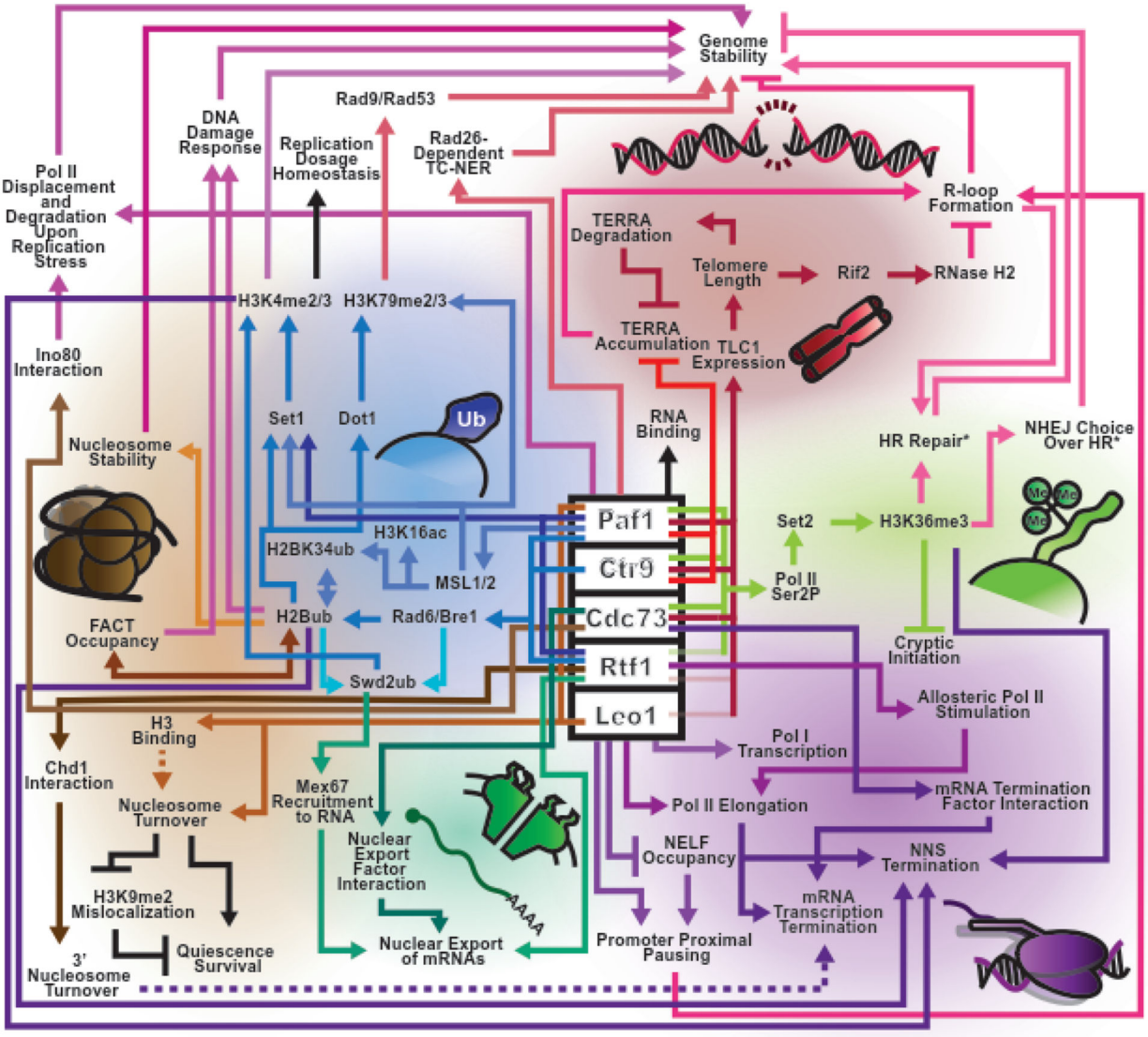

Figure 1. Network of Paf1C Functions.

Relationships between manually-curated, Paf1C-related physiological processes are depicted as either positive (arrow) or negative (bar). Functional domains are separated into related processes including nucleosome remodeling (brown), ubiquitylation (blue), genome stability (pink), RNA export (dark green), transcription (purple), H3K36me3 (light green), and telomere biology (maroon). Faded lines originating from Paf1C subunits indicate a weak, but present, regulatory relationship. Dotted lines indicate plausible but unproven relationships. The influence of H3K36me3 on DNA damage repair pathway is organism-specific (asterisks). See text for a detailed description of the interactions.

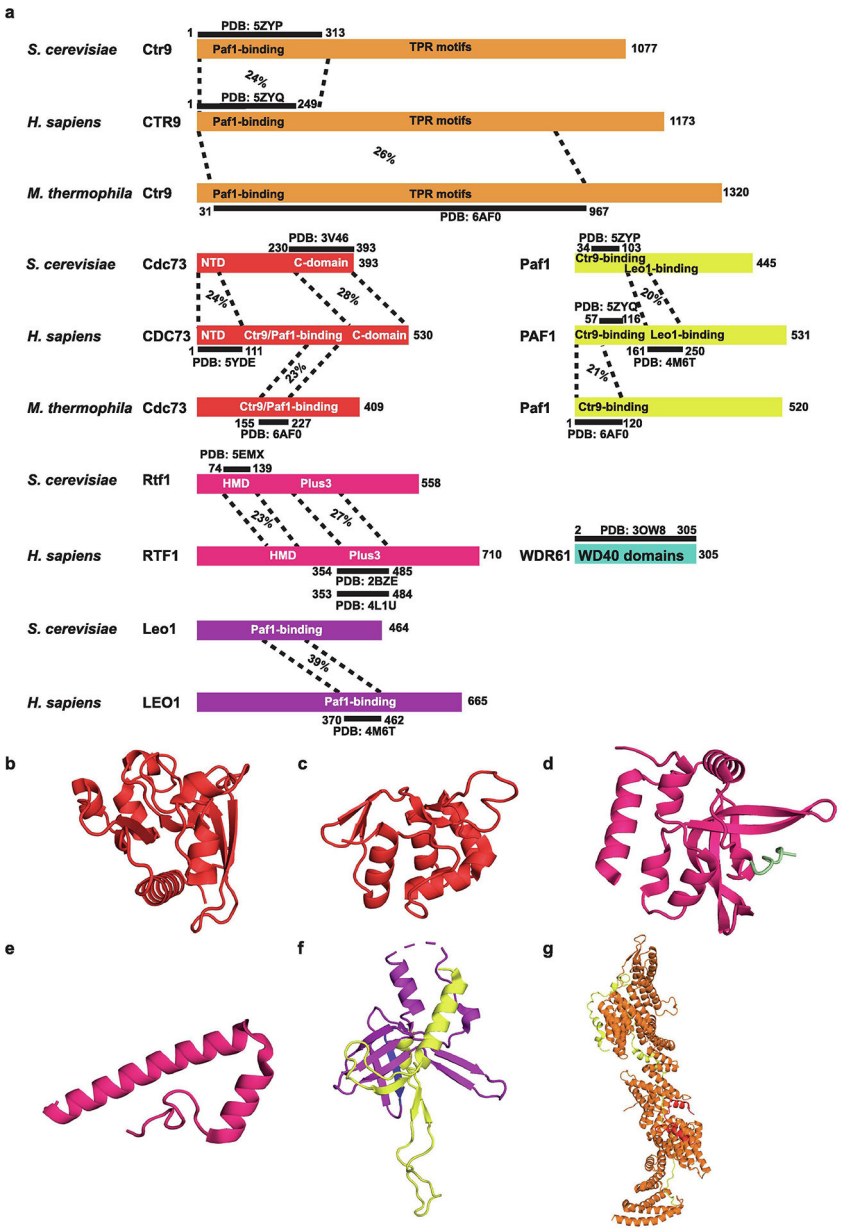

Figure 2. Structural analysis of domains and subcomplexes of Paf1C.

a) Diagram of Paf1C subunits of *S. cerevisiae*, *H sapiens*, and *M. thermophila*, highlighting the regions that have been structurally analyzed by X-ray crystallography or NMR. Black bars and numbering denote residues included in the constructs to observe the structure of individual proteins or subcomplexes. Dotted black lines mark areas of homology between *H. sapiens* and *S. cerevisiae* or *M. thermophila* proteins. Percent amino acid identity within the regions bracketed by the dotted lines is indicated. Protein sequences were obtained from Uniprot, aligned with Clustal Omega, and visualized in Jalview. Regions of homology were obtained from alignments, and percent ID values were obtained from pairwise alignments in Jalview. Available cryo-EM data are not indicated in this panel. b) The C-domain of *S. cerevisiae* Cdc73 (PDB: 3V46) [83]. c) The N-terminal domain (NTD) of human CDC73

(PDB: 5YDE) [90]. d) The Plus3 domain of human RTF1 in complex with an SPT5 CTR peptide (green) (PDB: 4L1U) [74]. e) The histone modification domain (HMD) of *S. cerevisiae* Rtf1 (PDB: 5EMX) [46]. f) The human PAF1-LEO1 heterodimer (with the linker construct shown in blue) (PDB: 4M6T) [57]. g) The *M. thermophila* PAF1-CTR9-CDC73 subcomplex (PDB: 6AF0) [94].

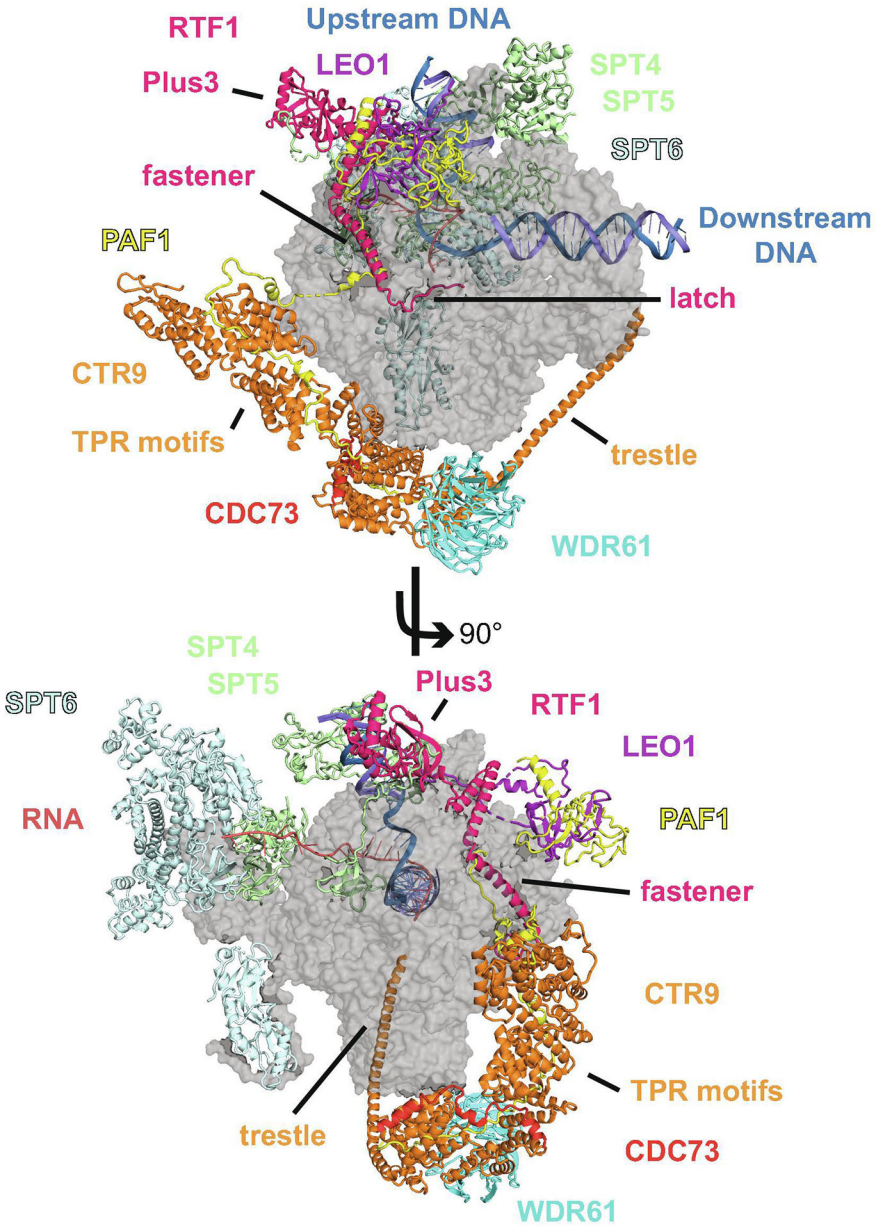

Figure 3. The organization and structure of PAF1C bound to a Pol II elongation complex.

Cryo-EM structure of human PAF1C, DSIF, and SPT6 in complex with *Sus scrofa* Pol II (PDB: 6TED) [63], highlighting the intermolecular interactions of PAF1C with other core members of the Pol II elongation complex. Full-length protein constructs of all six PAF1C subunits were included in the structure composition, though not all domains were visible. The PAF1-CTR9-CDC73 subcomplex, LEO1, full-length WDR61, and regions of RTF1 are visible in this structure.