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RNA polymerase II carboxy-terminal domain with multiple connections

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Abbreviations: IIo, hyperphosphorylated polymerase II; IIa, hypophosphorylated polymerase II; CDK, cyclin-dependent kinase; CTD, carboxy-terminal domain; CTDK1, CTD kinase complex 1; PIC, pre-initiation complex; pol II, RNA polymerase II; PPIase, peptidyl-propyl cis/trans isomerase; S, serine; SCP, small CTD phophatase; Tat, trans activator of transcription; TFIIH, general transcription factor IIH

Abstract

The largest subunit of eukaryotic RNA polymerase II contains a unique domain at its carboxy-terminus, which is referred to as the carboxy-terminal domain (CTD). The CTD is made up of an evolutionarily conserved heptapeptide repeat (YSPTSPS). Over the past decade, there has been increasing attention on the role of the CTD in transcription regulation in the view of mRNA processing and chromatin remodeling. This paper provides a brief overview of the recent progress in the dynamic changes in CTD phosphorylation and its role in integrating multiple nuclear events.

Keywords: carboxy-terminal domain kinase; chromatin; phosphorylation; histones; RNA polymerase II; RNA processing, post-transcriptional

Introduction

The largest subunit of eukaryotic RNA polymerase II (pol II) carboxy-terminal domain (CTD) consists of conserved heptapeptide repeats ($Y^1S^2P^3T^4S^5P^6S^7$) (Dahmus, 1996). Mammalian pol II CTD contains 52 repeats, whereas the yeast *Saccharomyces cerevisiae* CTD has 26-27. A deletion of the mouse, Drosophila, or yeast CTD is lethal. Therefore, the CTD is essential for the viability of an organism, even though the number of repeats can be reduced. Partial deletions of the CTD result in reduced transcription *in vivo*, and defective responses to various activators. The CTD acts as a platform to couple the mRNA metabolism and chromatin function to the transcription as it recruits various RNA processing/export and histone modifying factors to the transcription complex (Bentley, 2005; Buratowski, 2005; Phatnani and Greenleaf, 2006). This means that the CTD is very important for organizing various nuclear functions to acquire the proper regulation of gene expression. Those functions often depend on the CTD modification such as phosphorylation. Indeed, the CTD is rich in phospho-acceptor amino acid residues and undergoes reversible phosphorylation during the transcription cycle. Two forms of RNA pol II, which differ in the level of phosphorylation of the CTD, can be distinguished and are believed to have distinct functions in the transcription cycle; RNA pol IIa, with a hypophosphorylated CTD, is the form that assembles into the transcription initiation complexes, whereas pol IIo, with a hyperphosphorylated CTD is associated with the transcription elongation complexes. Phosphorylation occurs mainly at discrete serines (S) within the CTD repeats (S2, S5), which is then recognized by different proteins that interconnect the transcription to various nuclear metabolisms. Accordingly, serine phosphorylation is known as the 'CTD code', in a similar way that the 'histone code' refers to the histone modification (Buratowski, 2003).

CTD with the phosphorylation code

Earlier models based on a two-step transcription cycle, in which pol IIa was assembled at the promoter and pol IIo carried out transcription elongation, have evolved to one with a more complex CTD phosphorylation cycle. Different modified forms of pol II dominate different stages of transcription (Komarnisky *et al.*, 2000). Pol II assembled at the promoter is phosphorylated at the S5 of the CTD repeat through transcription factor IIH (TFIIH). The CTD is partially dephosphorylated at this position after it escapes into the elongation phase. As elongation proceeds, the level of phosphorylation of the CTD at the S2 increases and peaks near the 3' end of a gene (Figure 1). In accordance with the different locations and the timing of the modification, the serines in positions 2 and 5 are functionally

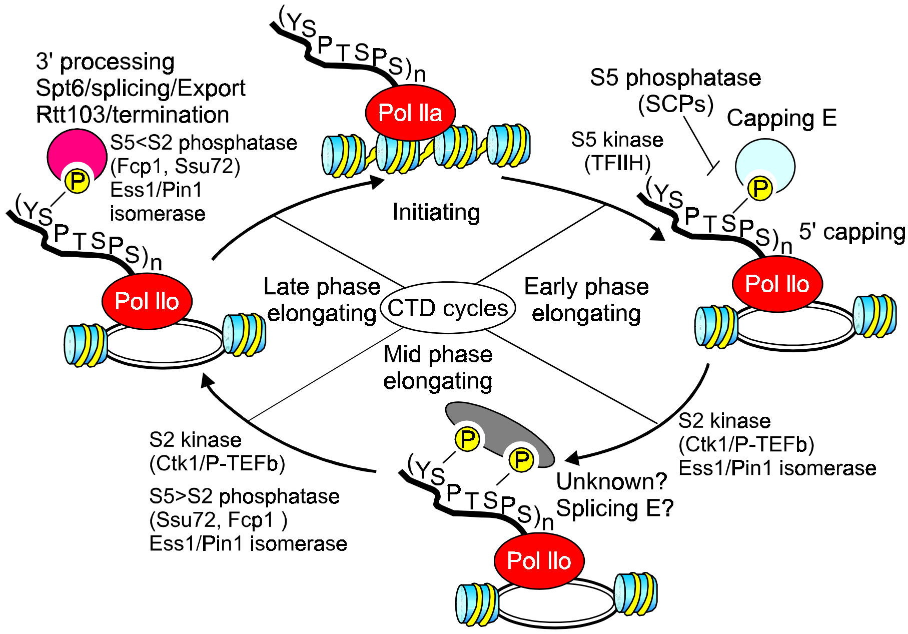

Figure 1. RNA polymerase II CTD phosphorylation cycle. RNA pol II with a heptapeptide repeat ($Y^1S^2P^3T^4S^5P^6S^7$) interacts with the DNA to initiate transcription. Upon phosphorylation by TFIIH at S5, the capping enzyme associates and waits until the 5' RNA is long enough to become exposed. Transition into the elongation phase by S5 phosphorylation might be inhibited by S5 phosphatase such as SCPs. As pol II travels downstream, Ctk1 (PTEF-b in human) starts to phosphorylate S2. In the meanwhile, S5 phosphatase, such as Ssu72, dephosphorylates S5, leaving S2 phosphorylated pol II during the processive elongation phase. S2 phosphatase such as Fcp1 counteracts Ctk1/P-TEFb to balance S2 phosphorylation. Phosphorylated S2 is recognized by 3' processing, splicing, termination, and exporting factors. Different serines with different levels of phosphorylation (along the repeats) might serve as a binding platform for various nuclear factors. As soon as pol II passes the poly(A) sites, Fcp1 removes S2 phosphorylation completely with the aid of Ssu72, which allow pol II to initiate another cycle. See the details in the text.

different (Phatnani and Greenleaf, 2006). CTD is phosphorylated by members of the cyclin-dependent kinase (CDK) family, which typically consists of a catalytic subunit and a regulatory cyclin subunit. These kinases phosphorylate distinct positions within the repeat to exert distinct functions (reviewed in Meinhart *et al.*, 2005).

The kinase subunit (Kin28) of yeast TFIIH, associated with Ccl1 cyclin, is a major S5 kinase. The TFIIH is essential for the efficient coupling of the mRNA 5' modification because the cotranscriptional recruitment of the capping enzyme and the placement of the 7-methyl guanosine cap on pre-mRNA is dependent on S5 phsophorylation (See below) (Komarnitsky *et al.,* 2000; Rodriguez *et al.,* 2000; Schroeder *et al.,* 2000). Whereas, the yeast kinase subunit (Ctk1) of the CTD kinase 1 complex (CTDK1) is a major S2 kinase. Ctk1 efficiently phosphorylates the CTD during a processive elongation phase (Cho *et al.,* 2001). The Cdk9 subunit of the mammalian elongation factor P-TEFb is functionally similar to Ctk1 (Price, 2000). Cdk9 has been shown to phosphorylate S2, which emphasizes the functional counterpart of Ctk1. However, its substrate specificity can be modified to favor S5 through an interaction with Tat *in vitro* (Zhou *et al.,* 2000). The temporal and spatial regulation of the kinase activity and the outcome of a specific combination of the phosphorylated serines all play important roles in regulating the function of the CTD.

Isomerization even multiplies the CTD code

Peptidyl-prolyl cis/trans isomerase (PPIase) catalyzes the rotation of the peptide bond on the amino-terminal side of proline residues, a step known to modulate the proper folding of newly synthesized proteins (Schiene and Fischer, 2000). Mammalian Pin1 and its yeast homolog, Ess1, are the most interesting PPIase implicated in the transcription. Pin1/Ess1 plays a role in cell cycle and has been implicated in transcription through the direct and preferential binding to the phosphorylated CTD (Morris *et al.,* 1999; Verdecia *et al.,* 2000). Pin1/Ess1 contains an N-terminal WW domain and a C-terminal PPIase domain. The WW domain is a small structural motif that functions as an interaction module to bind the proline-rich domains of a variety of signaling proteins (Sudol and Hunter, 2000). The same domain of Pin1/Ess1 is responsible for its interaction with the phosphorylated CTD. Genetic and biochemical studies in yeast have shown a possible linkage between the PPIase activity and transcription that brings Ess1 to be a secondary regulator to remodel the CTD code (Wu *et al.,* 2000; Wilcox *et al.,* 2004). In the present model, Ess1 with PPIase activity would bind the phosphorylated CTD through the WW domain in order to reconfigure the structure of the CTD through isomerization of the proline peptide bond (Buratowski, 2003). Because highly phosphorylated pol II is correlated with the transcript elongation, the binding of Ess1 may affect the function of the elongating pol II. In this scenario, the conformational change can affect the association and disassociation or the activity of many CTD-interacting proteins such as the 3' processing factors. Isomerization of the CTD can also provide a better substrate for Fcp1 CTD phosphatase to facilitate the recycling of pol II (Kops *et al.,* 2002). Therefore, Pin1/Ess1 has a potential to regulate the transcription by changing the CTD code.

Deciphering the CTD code

It is important to know what reads the CTD phosphorylation code in order to understand how it operates. This can be best answered by reviewing the proposed coupling mechanism between transcription and RNA processing. Over the past few years, many observations have contributed to the idea of the cotranscriptional processing of nascent RNA through the direct coupling to the transcription. The CTD has always played an important role in both targeting the RNA processing machinery and regulating their catalytic activity.

The cap structure is a characteristic of all RNA pol II transcripts and consists of an inverted 7-methyl guanosine cap that is linked to the first RNA residue by a 5'-5' triphosphate bridge. Capping is performed by a series of three enzymes; RNA 5'-triphosphatase, guanylyltransferase, and RNA (guanine-7) methyltransferase. The capping enzyme binds directly and specifically to the CTD of pol II through the Ceg1 subunit (yeast) or guanylyltransferase domain (in metazoan, as it is synthesized together with the RNA 5'-triphosphatase as a bifunctional polypeptide) when S5 is phosphorylated by TFIIH (Komarnitsky *et al.,* 2000; Schroeder *et al.,* 2000). Furthermore, the capping enzyme activity is stimulated by an interaction with the phosphorylated CTD, and in return, enhances early transcription. This is considered to be a mechanism that stimulates the extension of the capped RNA only, by coupling capping and the early transcription (Cho *et al.,* 1998; Ho and Shuman, 1999; Kim *et al.,* 2004a; Schroeder *et al.*, 2004).

In contrast to the capping, the splicing machineries contain consensus binding sites on the nascent RNA. Therefore, there is some controversy as to whether co-transcriptional splicing (splicing while transcription is ongoing) is required functionally or is simply linked mechanically (i.e. RNA is spliced independently of transcription) (Kornblihtt *et al.,* 2004). Chromatin immunoprecipitation analysis shows that the direct binding of the splicing machinery to the nascent RNA is responsible in a large part for the co-transcriptional splicing in yeast and mammals (Listerman *et al.,* 2006; Moore *et al.,* 2006; Tardiff *et al.,* 2006). However, CTD might also play a role by providing a platform for the splicing machinery and even regulate the choice of alternative exons by increasing the local concentration of proteins (de la Mata and Kornblihtt, 2006). Splicing factors including small nuclear ribonucleoprotein particles (snRNPs) and non-snRNP proteins such as the serine/arginine-rich (SR) protein family are associated with pol IIo but not with pol IIa (Mortillaro *et al.,* 1996; Kim *et al.,* 1997). The arginine-serine rich (RS) domain of the SR family protein is essential for recruitment to the phosphorylated CTD (Misteli and Spector, 1999). In yeast, the splicing factor, Prp40, has been reported to bind to the phosphorylated CTD (Morris and Greenleaf, 2000). In particular, mammalian Spt6 binds selectively to the phosphorylated S2 through the SH2 domain and couples hIws1 dependent mRNA splicing (Yoh *et al.,* 2007). Indeed, a phosphorylated CTD is required for the efficient splicing reaction (Bird *et al.,* 2004; Millhouse and Manley, 2005). RNA pol IIo stimulates the *in vitro* reconstituted splicing reaction of pre-mRNAs, while the addition of the phosphorylated CTD peptides inhibits this reaction (Du and Warren, 1997; Hirose *et al.,* 1999). This indicates that an elongating pol II with phosphorylated CTD is an active component of the splicing reaction. Like capping, pol II CTD can play an important role in splicing by regulating the efficiency and specificity of splicing as well as recruiting the machinery. However, the functional specificity of the two different serines is unknown.

Similar to splicing, the 3'-end processing of mRNA is affected in cells through a deletion of the pol II CTD or a loss of CTD phosphorylation, even though nascent RNA carries the consensus recognition sites (Fong and Bentley, 2001; Proudfoot *et al.,* 2002; Skaar and Greenleaf, 2002; Ahn *et al.,* 2004). 3'-end modifications of the pre-mRNA proceeds through two steps; endonucleolytic cleavage of the mRNA precursor followed by poly(A) addition to the cleavage product. CF1A, CF1B, and CFII in yeast, and the similar complexes, CstF, CPSF, CF1, and CF2 in higher eukaryotes, perform this function while polyadenylation is mediated by poly(A) polymerase in both. The 3'-end processing factors can bind to the CTD affinity column (McCracken *et al.,* 1997). Furthermore, several factors, including Pcf11, Pta1, and Rna14 show an apparent preference for binding to the phosphorylated CTD (Rodriguez *et al.,* 2000; Barilla *et al.,* 2001; Licatalosi *et al.,* 2002; Meinhart and Cramer, 2004). In particular, the yeast 3'-end processing factors appear to be recruited in time through the phosphorylation of S2 of the CTD when pol II approaches the 3'-end of a gene. Ctk1 is responsible for S2 phosphorylation and is in turn responsible for the selective binding of the 3'-end processing factors (Ahn *et al.,* 2004). Therefore, the S2-phosphorylated CTD can act as a platform for these factors. In addition to serving as a binding surface, both phosphorylated and non-phosphorylated CTD activate the cleavage reaction *in vitro*. The CTD might not be essential for the reaction but it certainly enhances the efficiency by coupling the two pathways (Hirose and Manley, 1998).

The 3'-processing signal elements in turn affect the efficiency of transcription termination. 3'-cleavage/polyadenylation and termination must be closely coupled because the poly(A) signals are required for proper transcription termination in mammals and yeast (Bauren *et al.,* 1998; Birse *et al.,* 1998). The connections among 3'-end processing, CTD phosphorylation, and termination were recently resolved by identification of the Rtt103 protein, which is a 3'-end mRNA processing factor that contains a CTD interacting domain. Rtt103 interacts with the CTD in a S2-phosphorylation dependent manner and recruits the 5'→3' RNA exonuclease that is responsible for the release of pol II from the DNA template (Kim *et al.*, 2004b; West *et al.,* 2004). In yeast, mRNA export is also linked to transcription via the TREX (transcription export) complex (reviewed in Aguilera, 2005). TREX is composed of the four-subunit complex, THO (Tho2, Hpr1, Mft1, and Thp2) and the evolutionally conserved RNA export proteins, Sub2 (UAP56 in human) and Yra1 (REF/Aly in human). Deletions of individual THO components lead to impaired transcription, transcription-dependent hyper-recombination, and mRNA export defect (Jimeno *et al.,* 2002; Strasser *et al.*, 2002). In addition, *SUB2* and *YRA1* mutants are synthetic lethal with THO mutants and over-expressed Sub2 suppresses the THO mutant phenotype (Fan *et al.,* 2001), which all supports the potential linkage of transcription elongation to mRNA export. In addition to genetic interaction with THO, Sub2/Yra1 are directly recruited to the actively transcribed regions via physical interaction with THO (Strasser *et al.,* 2002; Zenklusen *et al.,* 2002), suggesting a one-step biogenesis of export-competent mRNP while transcription is ongoing. However, the potential role of the pol II CTD and CTD phosphorylation in this process remains unclear. Recruitment of the TREX complex to transcribed genes is not dependent on the S2 kinase, Ctk1 in yeast (Ahn *et al.,* 2004), and the association of the human TREX complex to mRNA might be coupled to transcription indirectly through splicing (Masuda *et al.,* 2005). On the other hand, interestingly, Jones and colleagues show that mammalian Spt6 that selectively associates S2-phosphorylated CTD concomitantly recruits REF/Aly and UAP56 via Iws1 (Yoh *et al.,* 2007), suggesting an alternative mechanism of cotranscriptional coupling of mRNA export in mammalian system independently of THO or splicing, but depends on CTD phosphorylation. In summary, many aspects of the mRNA metabolism from the 5' capping to the export occur cotranscriptionally and are coordinated through transcription with the keyword of the pol II CTD or CTD phosphorylation.

The CTD code translated into the histone code

The transcription states are intimately linked to the chromatin states (Figure 2) (Gerber and Shilatifard 2003; Hampsey and Reinberg 2003; Saunders *et al.,* 2006). The basic element of chromatin, the nucleosome, consists of a 146 bp DNA wrapped around a histone octamer that is composed of two copies of H2A, H2B, H3, and H4 (Luger, 2003). The post-translational modification of the histones, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation regulate gene expression by controlling the accessibility of various transcription factors (Cheung *et al.,* 2000; Nathan *et al.,* 2003; Martin and Zhang, 2005). Among them, the histone H3 lysine (K) 4 and K36 are the most characterized methylation sites that have been implicated in active transcription. H3 K4 is methylated by the proteins of the Set1 family, while K36 is methylated by the proteins of Set2 (Gerber and Shilatifard, 2003). The profile of H3 K4 tri-methylation is strongly correlated to the distribution pattern of the pol II phosphorylated at S5 (Figure 2). This usually peaks at the promoter and 5' region of a gene, indicating a role in an early phase of transcription (Pokholok *et al.,* 2005; Millar and Grunstein, 2006). As expected from the distribution profile, the Set1 complex is associated with the S5 phosphorylated pol II (Ng *et al.,* 2003). H3 K4 mono- and di-methylation tend to spread out compared with the tri-methylation. On the other hand, H3

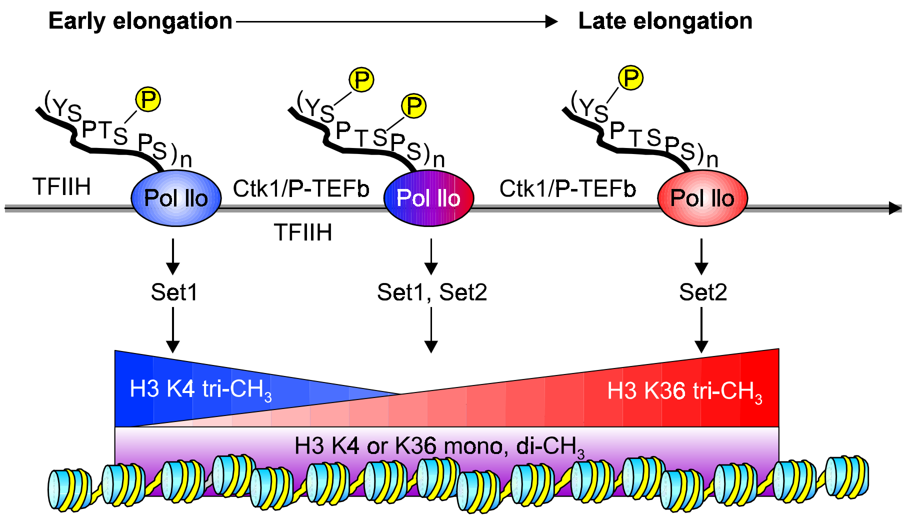

Figure 2. The CTD code translated onto the histone code. As polymerase transcribes DNA, the transcription states reflected by the CTD code leave a mark on the chromatin by changing the histone N-termini. The chromatin of an actively transcribed gene is methylated at histone H3 K4 (tri-CH_3) with the typical pattern of a peak around the promoter and the 5' of a coding region, while H3 K36 (tri-CH_3) is methylated with an opposite pattern of a peak toward the 3' of a coding region. Mono- and di-methylated H3 K4 or K36 (mono-, di-CH_3) are linked evenly along a gene compared with the tri-methylated form. Phosphorylated S5 and S2 play important roles in coupling of histone methylation through a specific interaction with Set1 and Set2, respectively. The CTD phosphorylation states are determined by the relative prevalence of the different kinases and phosphatases.

K36 tri-methylation has been observed throughout the coding region with an increase toward the 3' region of an actively transcribed gene. In contrast to Set1, the recruitment of Set2 depends on S2 phosphorylation (Krogan *et al.,* 2003a; Xiao *et al.,* 2003). Therefore, differently phosphorylated CTD by TFIIH and Ctk1 is responsible for the characteristic distribution of H3 K4 and K36 methylation. In addition to direct recruitment of Set2, Ctk1 restricts the spread of H3 K4 tri-methylation in the coding region (Xiao *et al.,* 2007). The extent of methylation on each residue is further regulated by the elongation complex, Paf1 (Krogan *et al.,* 2003b). In addition, the Rad6/Bre1 complex dependent mono-ubiquitination of H2B on K123 or the BUR kinase complex is important for the di- and tri-methylation of histone H3 K4 (Shahbazian *et al.,* 2005; Laribee *et al.,* 2005; Wood *et al.,* 2005). Overall, the nature of the RNA polymerase II complex engaged in various stages of transcription is reflected onto the chromatin through the histone code, which is translated cotranscriptionally from the CTD code. Although the molecular consequences linking transcription and chromatin modification are unclear, the pol II CTD and transcription factors play an important role in coupling these pathways.

Conclusion

The phosphorylation of the CTD at S5 by TFIIH or at S2 by Ctk1 is essential for the coupling of RNA processing or the chromatin function to the transcription. The phosphorylation of serine sites of the CTD in various combinations is one way of creating multiple connections that make transcription as a center of gene expression and chromatin function. Further insight into the coupled nuclear events as well as the role of these connections will be possible when a full list of the proteins that read and write the CTD code is revealed.

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