Transcription: another mark in the tail

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The C-terminal domain (CTD) of the largest RNA polymerase II (RNAPII) subunit undergoes dynamic phosphorylation to support transcription-associated events and drive the transcription cycle. In mammalian cells, it comprises 52 repeats of the heptapeptide sequence Tyr₁–Ser₂–Pro₃–Thr₄–Ser₅–Pro₆–Ser₇. While important functions for Ser₂-, Ser₅-, and Ser₇-phosphorylation have previously been described, a new report in *The EMBO Journal* now suggests an unexpectedly crucial role for Thr₄ phosphorylation as well.

The three eukaryotic RNA polymerases (RNAPI, RNAPII, and RNAPIII) have a very similar overall configuration and even share several subunits. The two largest subunits, which together form the active sites of the respective enzymes, are also highly related (Cramer *et al*, 2008). However, one feature in particular sets the mRNA-producing polymerase, RNAPII, apart from its relatives: its C-terminal domain (CTD). Even though the CTD is dispensable for *in vitro* transcription using purified transcription factors, it plays a crucial role *in vivo* (Buratowski, 2009; Perales and Bentley, 2009). The form of RNAPII that is recruited to gene promoters has a largely unphosphorylated CTD, but as transcription progresses, Ser₅ and Ser₂ are phosphorylated and dephosphorylated sequentially to drive the transcription cycle and regulate co-transcriptional events. For example, Ser₅ phosphorylation by the general transcription factor TFIIF results in the dissociation of the initiation-specific Mediator complex from RNAPII, helping release polymerase from the promoter. Ser₅ phosphorylation also helps recruit specific factors to the transcribed gene, including the mRNA 5'-end capping enzyme, chromatin-modifying factors, and mRNA splicing factors (Buratowski, 2009; Perales and Bentley, 2009). As the transcription cycle proceeds into elongation, Ser₅ phosphorylation is removed by CTD phosphatases, and another mark, Ser₂ phosphorylation, is added in its place. Even though Ser₂ phosphorylation is the predominant mark on the CTD of elongating polymerases, it often overlaps with Ser₅ phosphorylation across the coding region of an average gene (Figure 1). Among its known functions, Ser₂ phosphorylation plays an important role in attracting histone-modifying enzymes, as well as in mRNA 3'-end processing (Buratowski, 2009; Perales and Bentley, 2009).

Although the majority of CTD research has focused on Ser₂ and Ser₅ phosphorylation, a full five out of the seven amino acids in the consensus CTD repeat are potential

phospho-receptor sites (Tyr₁, Ser₂, Thr₄, Ser₅, and Ser₇). Indeed, Tyr₁ and Ser₇ are also targets of phosphorylation. Ser₇, which is phosphorylated by TFIIF, is relevant for mammalian snRNA 3'-end processing, at least partly via recruitment of the Integrator complex (Egloff *et al*, 2010 and references therein). The physiological role of Tyr₁ phosphorylation is presently unclear, but this modification appears to be dependent on c-Abl and the related Arg kinase (Baskaran *et al*, 1993; Baskaran *et al*, 1997). This leaves Thr₄ as the only potential phosphorylation site that has scarcely been studied. Although Thr₄ is phosphorylated in fission yeast (Sakurai and Ishihama, 2002), its relevance was put into question by the finding that it can be mutated in both budding and fission yeast RNAPII without affecting viability (Stiller and Cook, 2004; Schwer and Shuman, 2011). However, two recent papers, one of them published in this issue of *The EMBO Journal*, have now investigated the role of Thr₄ phosphorylation in higher eukaryotes and concluded that this mark plays an important role for gene expression in these cells (Hsin *et al*, 2011; Hintermair *et al*, 2012).

Hintermair *et al* (2012) tested the importance of Thr₄ using a human cell line expressing a version of RPB1/POLR2A that is insensitive to the RNAPII inhibitor α -amanitin. In this cell line, the endogenous Rpb1 subunit of RNAPII is degraded upon treatment with the drug, forcing the cell to survive on the α -amanitin-insensitive version of the protein (Gerber *et al*, 1995). Mutations can then be introduced in the gene

CTD phosphorylation in an average eukaryotic gene

Figure 1 CTD phosphorylation changes during the transcription cycle. Please note that the graphs are idealized and that Ser2 and Ser5 phosphorylation profiles represent a mixture of result from yeast and mammalian cells.

encoding this Rpb1 version to test their effect on transcription and cell viability. Surprisingly, Hintermair *et al* find that Thr₄ mutation to alanine is as detrimental as mutation of Ser₂, or Ser₅, suggesting an important role for this residue and its modification in transcription. In related work, Hsin *et al* (2011) performed a similar experiment in chicken DT40 cells and found that Thr₄ mutation is also lethal in these cells. Somewhat surprisingly, however, Hsin *et al* (2011) did not find evidence for general transcription defects in chicken cells. In contrast, the data by Hintermair *et al* (2012) indicate that Thr₄ plays a general and important role in transcript elongation in mammalian cells.

The pattern of Thr₄ phosphorylation in human cells is remarkably similar to that of Ser₂ phosphorylation: its levels are low at promoters but rise through the coding region to peak downstream of the gene, before dropping to very low levels again (see Figure 1). The overlap with Ser₂ phosphorylation is also apparent at other levels. First, virtually all Thr₄ phosphorylated polymerases are also Ser₂ phosphorylated, while the reverse is not true. Second, Hintermair *et al* (2012) found that replacing Ser₂ with non-phosphorylatable alanine abolished Thr₄ phosphorylation *in vivo*. This could suggest that Ser₂ phosphorylation is a prerequisite for subsequent Thr₄ phosphorylation and might explain the observation of Hsin *et al* (2011) that CDK9 (the Ser₂ kinase) is required for Thr₄ phosphorylation in DT40 cells. In mammalian cells, Hintermair *et al* (2012) present a convincing case for Polo-like kinase 3 (Plk3) being the enzyme responsible for Thr₄ modification; however, the precise functional relationship between CDK9/Plk3 and Ser₂P/Thr₄P remains to be clarified. Of equal importance is the finding by Hintermair

et al (2012) that the distribution of RNAPII across genes is significantly affected by Thr₄ mutation. Recruitment of the mutated polymerase to promoters is largely unaffected, but a substantial increase in RNAPII density is observed immediately downstream from the promoter, suggesting an elongation defect. Indeed, fewer polymerases appear to reach the end of genes in mutant cells compared with wild type. Since transcript elongation kinetics can greatly affect co-transcriptional events such as mRNA processing (Perales and Bentley, 2009), this might explain the defects in histone mRNA 3'-end processing observed when Thr₄ is mutated in DT40 cells (Hsin *et al*, 2011).

The phosphorylation marks on the three serines of the CTD all result in the specific recruitment of factors that affect transcription-associated events. This may very well also be true for Thr₄. However, the close relationship with Ser₂ phosphorylation, and the proximity of the Ser₂ and Thr₄ marks in the heptapeptide repeat, also opens the intriguing possibility that Thr₄ may serve as a 'dissociation mark' that dislodges Ser₂-binding factors. Such factors might include ones that need to be restricted to associating with RNAPII only temporarily.

Together, these recent analyses of Thr₄ roles in vertebrate cells have opened a new chapter in the study of the RNAPII CTD, opening the door to new and important questions about the mechanistic basis for the intriguing effects of Thr₄ phosphorylation on transcription.

Conflict of interest

The author declares that he has no conflict of interest.

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