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New link between the RNA polymerase II-CTD and replication stress

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ABSTRACT

Conflicts between transcription and replication are a major source of replication stress. Our recent findings show that proper dephosphorylation of Serine 5 in the carboxy-terminal domain (CTD) of DNA-directed RNA polymerase II subunit RPB1 is needed to prevent such conflicts in human cells.

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Replication stress promotes genome instability and can be exploited in cancer treatment. Detailed knowledge of the causes and consequences of replication stress is therefore relevant both to understand the development and to improve the treatment of cancer. Transcription-replication conflicts (T-R conflicts) are a common source of replication stress, but how the transcription machinery is regulated to prevent such conflicts has until now remained poorly understood. In our recent work,[1] we identify a regulatory pathway that promotes degradation of the largest core component of RNA polymerase II, DNA-directed RNA polymerase II subunit RPB1 (hereafter referred to as RNAPII), on chromatin and suppresses T-R conflicts in human cells. Since degradation of RNAPII on chromatin occurred both in the absence and presence of exogenous replication stress, our work suggests that continuous turnover of RNAPII on chromatin is required to prevent T-R conflicts even during normal cell growth.

Notably, RNAPII degradation has previously been proposed to promote the overall efficiency of transcription by removing stalled RNAPII complexes.[2] Our work further extends this by showing that RNAPII degradation is also required to prevent T-R conflicts. Furthermore, we identify an inhibitory role for Serine 5 phosphorylation (pS5) in the Carboxy-terminal domain (CTD) in RNAPII degradation of chromatin. A similar role for pS5 in RNAPII degradation was previously observed in yeast.[2] At the time, pS5 was proposed to facilitate the specific degradation of elongating RNAPII, as pS5 was believed to be primarily associated with promoter proximal regions.[2] However, as pS5 was later shown to be located throughout the gene body,[3] the reason pS5 inhibits RNAPII degradation is currently unclear. Interestingly, pS5 is particularly enriched at splice sites,[3] which are sites of RNAPII pausing.[4] One possibility is therefore that pS5 may protect RNAPII from unwanted degradation during transcriptional pausing.

Our study focuses on the protein phosphatase complex containing the serine/threonine-protein phosphatase 1 catalytic subunit (best known as PP1) and PP1 regulatory subunit 10 (best known as PP1 nuclear targeting subunit (PNUTS)). PP1 is involved in many cellular processes, and specific functions are mediated by regulatory proteins such as PNUTS.[5] The only established substrate of PNUTS-PP1 is pS5 in RNAPII.[6] Moreover, PNUTS is enriched in promoter-proximal regions, at splice sites and termination sites,[7] which are frequently associated with RNAPII pausing.[4] We show that a strong binding partner of PNUTS, WD repeat-containing protein 82 (WDR82), is also required for pS5 dephosphorylation in live cells.[1] Moreover, depletion of either PNUTS or WDR82 caused less degradation and higher stability of RNAPII on chromatin, resulting in T-R conflicts. We hypothesized that reduced pS5 dephosphorylation was causing the effects on RNAPII stability and T-R conflicts. Supporting this, wild-type PNUTS, but not a PP1 binding deficient mutant, rescued the effects on DNA replication and RNAPII chromatin stability after depletion of endogenous PNUTS. Furthermore, depletion of a different pS5 RNAPII phosphatase, RNA polymerase II C-terminal domain phosphatase SSU72 (best known as SSU72), also stabilized RNAPII on chromatin and caused replication stress, thus supporting that pS5 is the relevant substrate that stabilizes RNAPII on chromatin.[1]

Underscoring the intricate role of RNAPII pS5 phosphorylation is the observation that it also plays a role in signaling during the DNA damage response. Indeed, our previous work on PNUTS-PP1 suggests that RNAPII pS5 promotes signaling

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via the apical DNA damage kinase, ataxia telangiectasia, and Rad3-related (ATR).[8] At first glance the connection between our previous and current findings seems obvious. ATR is well known to respond to replication stress, and therefore, T-R conflicts caused by RNAPII pS5 could be leading to ATR signaling. Although the latter is likely a contributing factor, ATR signaling after depletion of PNUTS was also observed in G1 phase, arguing that ongoing DNA replication is not a strict requirement. In addition, ATR signaling after depletion of PNUTS was higher than expected compared to similar levels of replication stress caused by hydroxyurea.[8] Furthermore, replication stress is believed to activate ATR by leading to single stranded DNA coated with replication protein A (RPA).[9] However, the high ATR signaling after depletion of PNUTS was not reduced by co-depletion of replication protein A 70 kDa DNA-binding subunit (RPA70), an essential subunit of the RPA complex. The high ATR signaling after PNUTS depletion therefore appears to be non-canonical. Interestingly, ATR activity after depletion of PNUTS was strongly reduced by co-depletion of cell division cycle 73 (CDC73), a phospho-CTD binding protein and component of the polymerase associated factor 1 (PAF1) transcription complex.[8] Furthermore, in our new work, we found that replication stress and RNAPII chromatin stability after depletion of PNUTS or its interacting partner WDR82 was also dependent on CDC73.[1] CDC73 may therefore contribute to ATR activity by stabilizing S5 phosphorylated RNAPII on chromatin.

Altogether, our results suggest that the phosphorylation status of the RNAPII CTD plays a key role in the regulation of both ATR activity[8] and replication stress[1] (Figure 1). In addition to its important roles in the regulation of transcription and mRNA processing,[10] the RNAPII CTD thus also appears to act as a signaling platform in genome maintenance.

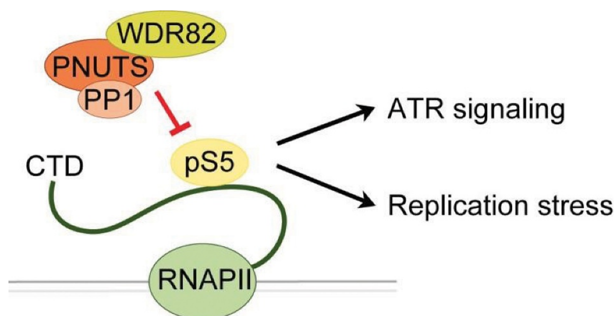

Figure 1. Model for how the RNA polymerase II-CTD acts as a signaling platform in genome maintenance. Serine 5 phosphorylation (pS5) in the carboxy-terminal domain (CTD) of DNA-directed RNA polymerase II subunit RPB1 (best known as RNAPII) promotes replication stress and signaling via the apical DNA damage kinase ataxia telangiectasia and Rad3-related (ATR). The phosphatase complex, consisting of serine/threonine-protein phosphatase 1 catalytic subunit (best known as PP1), PP1 regulatory subunit 10 (best known as PP1 nuclear targeting subunit (PNUTS)) and WD repeat-containing protein 82 (WDR82), dephosphorylates pS5 in the RNAPII CTD and thereby suppresses ATR signaling and replication stress in human cells. Note that ATR signaling via pS5 in the RNAPII CTD does not strictly require replication. See main text for details.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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