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Activation of P-TEFb by RBM7: To Live or Let Die

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In this issue of *Molecular Cell*, Bugai et al. (2019) unveil that a key step of the pro-survival cellular response to a genotoxic attack is the activation of P-TEFb by RBM7. This crucial step triggers RNA polymerase II release from promoter-proximal pausing and expression of DNA damage response genes.

The dynamic regulation of transcription by RNA polymerase II (Pol II) is a fundamental cellular response to environmental and developmental cues. Genome-wide studies have demonstrated that such regulation may occur at various stages after transcription initiation. In promoter-proximal pausing, Pol II is paused after transcription of 20–100 nucleotides and is released into productive elongation in a highly regulated stop-and-go movement (Jonkers and Lis, 2015). Pol II pause release requires the activity of a CDK-Cyclin complex called P-TEFb (positive transcription elongation factor b), made up of the catalytic subunit cyclin-dependent kinase 9 (CDK9) and its regulatory cycT1 or cycT2 subunit (Price, 2000). Upon its recruitment at target gene promoters, P-TEF-b phosphorylates serine 2 residues within the C-terminal domain of the largest Pol II subunit RPB1, as well as the negative transcription factor NELF and the SPT5 subunit of DSIF, leading to the release of RNA Pol II from proximal promoter pausing to elongation (Gressel et al., 2017). P-TEFb dynamically interacts with various partners to form several complexes, including the 7SK small nuclear ribonucleoprotein (snRNP)-associated P-TEFb (7SK–P-TEFb). Ample evidence indicated that this plasticity regulates P-TEFb activity. For instance, the 7SK small nuclear RNA and the three RNA-binding proteins (HEXIM1, MePCE, and LARP7) inhibit CDK9 kinase activity, and activation of P-TEFb takes place following its release from the complex (Michels and Bensaude, 2018). A recent study by Barboric and colleagues (Bugai et al., 2019) elegantly reports that activation of P-TEFb by the RNA-binding motif protein 7 (RBM7) protein is a novel key step taking place following a genotoxic attack, to express crucial DNA damage response (DDR) genes. Interfering with this activation leads to cell apoptosis after a genotoxic attack (Figure 1).

Up to this point, it was known that a cellular stress induces the activation of several protein phosphatases, including PP1α that dephosphorylates threonine 186 of the CDK9 T loop, leading to the release of P-TEFb from the 7SK snRNP complex and its activation (Chen et al., 2008). Here, the compelling findings of Barboric and colleagues reveal that the safe has more than one lock. Additional steps are required for the activation of P-TEFb implicating RBM7, a protein of the Nuclear EXosome Targeting complex (NEXT) involved in ribonucleolysis. Upon a genotoxic stress generated by UV irradiation or 4-NQO treatment, the $p38^{MAPK}$-MK2 pathway triggers the phosphorylation of RBM7 (Borisova et al., 2018). Based on interaction assays, Barboric and colleagues propose that the phospho-RBM7 binds to the core of 7SK snRNP through direct association with the stem loop 3 of 7SK, as well as with MePCE and LARP7. The formation of the RBM7-containing complex would mediate conformational changes and inhibit the LARP-7-dependent sequestration of HEXIM1 and P-TEFb, leading consequently to their release from the 7SK snRNP. The authors next show that the RBM7-dependent release and activation of P-TEFb allows a transcriptional induction of small coding and non-coding RNA genes involved in DDR. This smart model and the presented data beg several questions and some observations.

Regardless of the stimulus, the mechanisms of activation of P-TEFb appear quite similar (i.e., its release from the 7SK snRNP complex). It remains, however, that active P-TEFb is recruited to different specific promoters depending on a given stimulus, and that we have no clue as to how the targeting of these specific genes takes place. In DDR, the genes appear always very short, coding for small proteins or small ncRNA. This may prevent Pol II from encountering DNA damage during the transcription of these genes after a genotoxic stress. This feature is certainly important to allow the expression of these specific genes in a context in which a genotoxic attack leads to the repression of the majority of the genes while waiting for the repair of the genome. Whether or not the chromatin context or the proteins present when Pol II is pausing are different at these short genes is not known but may explain a differential recruitment of P-TEFb to these promoters after a genotoxic attack.

Consistent with this, we can imagine a role for RBM7 in the transcription of DDR genes apart from its involvement in P-TEFb release from 7SK snRNP. Barboric and colleagues observe the association of RBM7 with CDK9 and RNAPII phosphorylated on serine 2, suggesting that RBM7 could co-occupy promoter-proximal regions with P-TEFb, helping release of paused Pol II. In that respect, the behavior of the other subunits of the NEXT complex is not very clear. It seems that they are also present in the core of 7SK snRNP but are not involved in the P-TEFb activation. Is it linked that the phosphorylation of RBM7 would inhibit its implication in exosome? In other words, does the NEXT complex possess two independent

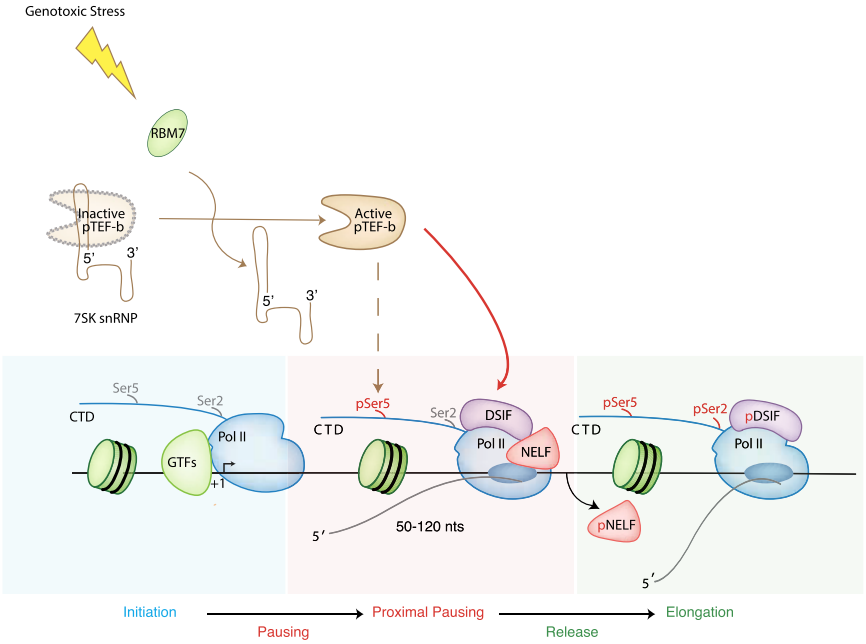

Figure 1. The Model Proposed by Barboric and Colleagues Involves the Activation of RBM7 by a Genotoxic Stress

Active RBM7 triggers the release of inactive P-TEFb from the 7SK–P-TEFb complex. The recruitment of active P-TEFb is mediated, among others, by the recognition of the phosphorylated serine 5 of the carboxyl-terminal domain (CTD) of the RPB1 subunit of Pol II. Pol II pause release is achieved by P-TEFb-mediated phosphorylation of CTD at serine 2, as well as NELF and DSIF complexes. Following phosphorylation, NELF dissociates from paused Pol II and DSIF helps Pol II in elongation.

functions depending on RBM7 phosphorylation state, and is the whole complex involved in the targeting of DDR genes after genotoxic stress? Alternatively, we cannot exclude the possibility that the association of RBM7 to CDK9/Pol II in elongation is used for exosome targeting of DDR RNAs after DNA has been repaired (Lubas et al., 2015).

Regardless, it must also be kept in mind that the recruitment of P-TEFb to paused Pol II can follow two different models. In addition to the release of P-TEFb from the 7SK snRNP that takes place before its recruitment to paused Pol II (the canonical model), 7SK–P-TEFb can in some cases be recruited to promoters before the activation of P-TEFb (McNamara et al., 2016). This model involves other factors such as the KAP1 protein (Trim28) that would recruit 7SK–P-TEFb to promoters containing paused Pol II. Which model is used in the activation of the DDR genes is not known, but the mode of recruitment could specify the genes that are activated by P-TEFb after a given stimulus.

Remarkably, a picture emerges from this study and others that the release of P-TEFb from 7SK snRNP after a genotoxic stress is tightly controlled by several signal pathways, such as dephosphorylation of CDK9 by PP1a or its takeover by RBM7. The locking of P-TEFb activation with several keys appears crucial to prevent the inappropriate release of the paused Pol II that may seal the fate of the cell without possible return.

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