Chaperoning RNA Polymerase II through repressive chromatin

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The histone chaperone Spt6 is known to facilitate transcriptional elongation while preventing spurious initiation of transcription within the coding region of genes. A recent publication in *The EMBO Journal* (Wang *et al*, 2013) identifies an additional mechanism by which Spt6 promotes gene expression by demonstrating that it tethers the histone demethylase UTX/KDM6A to the elongating RNA Polymerase II, thereby enabling the removal of repressive H3K27me3 marks within developmentally important muscle genes.

During myogenesis, the expression of muscle-specific genes is regulated by an antagonism between Polycomb (PcG) and Trithorax (TrxG) group proteins, which mark nucleosomes with transcriptionally repressive (H3K27me3) and permissive (H3K4me3 and H3K36me3) histone modifications, respectively. In proliferating myoblasts, muscle-specific genes are maintained in a repressed state through H3K27-trimethylation catalysed by the PcG protein Ezh2 that is recruited to the appropriate genes via the transcription factor YY1 (Carette *et al*, 2004). Upon differentiation, these genes undergo two temporally distinct stages of H3K27-demethylation mediated by the TrxG protein UTX/KDM6A, which permits their transcriptional activation

(Seenundun *et al*, 2010). In the first stage, UTX is targeted to the regulatory region of muscle-specific genes by the sequence-specific transcription factor Six4, leading to a localized removal of H3K27-trimethylation (Seenundun *et al*, 2010). This is thought to result in the formation of an active enhancer marked by H3K4me1/H3K27ac (Herz *et al*, 2012). In the second stage, UTX becomes associated with the elongating RNA Polymerase II (RNA Pol II), leading to the removal of H3K27me3 marks within the gene body allowing high expression levels (Seenundun *et al*, 2010). However, the mechanism by which UTX interacts with the elongating RNA Pol II had not been established thus far.

In this issue of *The EMBO Journal*, Wang *et al* (2013) resolve this question by demonstrating that UTX associates with the elongating RNA Pol II through an interaction with the transcription elongation factor Spt6. Spt6 is known to directly bind to the elongating form of RNA Pol II phosphorylated on Serine 2 (Yoh *et al*, 2007), and has previously been shown to augment the rate of transcriptional elongation (Ardehali *et al*, 2009). Furthermore, Spt6 is known to be required for expression of MyoD in somites of the developing zebrafish (Kok *et al*, 2007). Performing genome-wide ChIP analysis in myoblasts, Wang *et al* (2013) observe that Spt6

Figure 1 Spt6 enhances transcriptional elongation by tethering UTX/KDM6A to RNA Pol II. Wang *et al* (2013) demonstrate that Spt6 is essential for the expression of developmentally regulated muscle genes, as it tethers the histone demethylase UTX (KDM6A) to the elongating polymerase to ensure efficient removal of repressive H3K27me3 marks. Concurrent with the removal of H3K27me3, Spt6 is also required for accumulation of SetD2-mediated H3K36me3 marks within the gene body. As H3K36me3 has been shown to act as a docking site for the PCL1/3 components of the PRC2 complex, a temporary condensing of nucleosomes through a transient H3K27me3 mark could be envisaged. This repressive mark would then be quickly removed upon the arrival of the queued elongating RNA Pol II molecules traversing the gene. Therefore, Spt6 may modulate an on-going antagonism between UTX and PRC2 in the gene body to ensure efficient transcriptional elongation of transcripts initiated from the gene promoter, while preventing spurious transcription from initiating at cryptic sites within the gene.

and UTX localize to a largely overlapping set of genes in a manner that is inversely correlated with the presence of the repressive H3K27me3 mark. In addition, knockdown of Spt6 results in decreased association of UTX with muscle gene bodies with a concomitant increase in H3K27me3 marks and repression of transcription. Thus, Spt6 plays an essential role in tethering UTX to RNA Pol II to permit transcriptional elongation. These findings are reminiscent of recent work demonstrating that the related H3K27me3 demethylase JMJD3/KDM6B also associates with Spt6 to modulate transcription elongation in leukaemic cells (Chen *et al*, 2012). While it was initially proposed that the ability of JMJD3 to modulate transcriptional elongation could be a key difference between the two H3K27me3 demethylases (Chen *et al*, 2012), work from the Sartorelli group now establishes that UTX and JMJD3 share this key transcriptional regulatory function (Wang *et al*, 2013). Importantly, the need for Spt6 (and consequently UTX) to regulate expression of muscle genes can be overcome by a loss of the PcG protein Ezh2, demonstrating the importance of the Spt6/UTX complex in antagonizing the inhibitory function of PcG proteins to mediate transcriptional elongation (Wang *et al*, 2013).

Interestingly, JMJD3 has also been shown to interact with the H3K36 methyltransferase SetD2 (Chen *et al*, 2012), which mediates H3K36-trimethylation in the body of transcribed genes. While the association of Spt6 and UTX with SetD2 was not examined in myoblasts, loss of Spt6 leads to a decrease in H3K36me3 accumulation within muscle genes (Wang *et al*, 2013). This would suggest that an ability to associate with additional components of the transcriptional elongation machinery is likely conserved between UTX and JMJD3. The correlation between UTX/Spt6 and H3K36 trimethylation is particularly intriguing in light of the recent finding that the Tudor domain of the PcG proteins Polycomb-Like 1 and 3 (PCL1/PCL3) recognizes H3K36-trimethylated

nucleosomes and targets the Polycomb complex to transcribed genes (Cai *et al*, 2013). These results raise the exciting possibility that TrxG and PcG proteins may be acting antagonistically to ensure the integrity of gene transcripts by preventing spurious transcription (Figure 1). In such a model, SetD2-mediated H3K36-trimethylation would recruit PCL1/3-containing Polycomb repressive complex 2 (PRC2) to transcribed genes, resulting in H3K27-trimethylation behind the elongating RNA Pol II to create a condensed chromatin that is refractory to transcription initiation. However, these H3K27-trimethylated nucleosomes would be very short-lived as a trailing RNA Pol II would quickly displace PRC2. UTX/KDM6A would then remove the repressive H3K27me3 mark, while Spt6 acts to displace the impeding nucleosome to permit further elongation by RNA Pol II. Such a model is consistent with the previous finding that Spt6 prevents the use of cryptic promoter elements within genes (Kaplan *et al*, 2003).

In conclusion, the findings of Wang *et al* (2013) provide exciting new insight into the mechanism through which UTX tethers to the elongating RNA Pol II for H3K27 demethylation within the coding region of genes. Having established that an on-going antagonism between TrxG and PcG is modulating RNA Pol II elongation at muscle genes, it will be interesting to determine whether UTX and PRC2 are responsible for mediating Spt6-dependent inhibition of cryptic transcription initiation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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