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CHROMATIN

The chemical brothers: nucleosomes and transcription

The positioning of nucleosomes along the DNA with regard to various genetic elements is thought to affect transcription by competing with transcription factors for binding DNA. Using a high-resolution chemical approach to map nucleosomes, Voong *et al.* provide new insights into the interplay between nucleosomes, transcription and splicing.

Looking to improve on the accuracy of the common nucleosome-mapping method MNase-seq, the authors developed a genome-wide nucleosome-mapping approach that determines nucleosome centre (dyad) positions at nucleotide resolution based on chemical cleavage of the DNA. The method requires substituting Ser47 of histone H4, which flanks the nucleosome centre with a Cys residue ($H4^{S47C}$), which can be covalently bound by a copper-chelating compound. The copper ions direct cleavage of nucleosome DNA near the dyad by hydroxyl radicals, and the resulting DNA fragments are subjected to deep sequencing.

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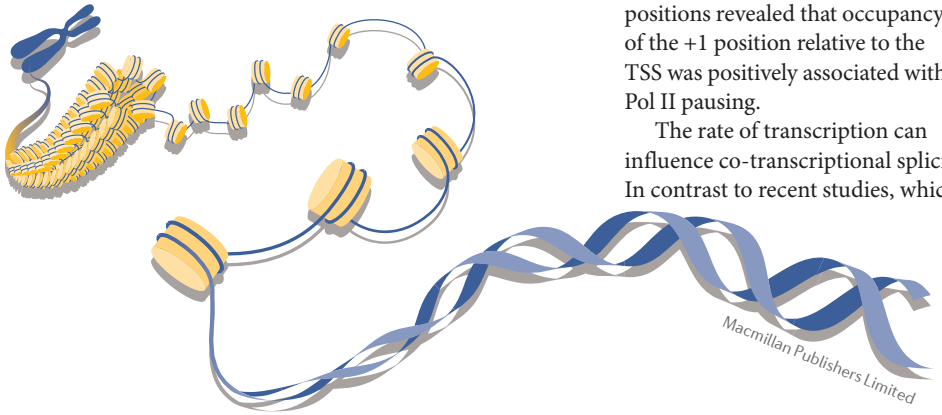

The authors substituted most of the endogenous H4 proteins in mouse embryonic stem (ES) cells with $H4^{S47C}$. MNase-seq nucleosome maps generated in $H4^{S47C}$ and wild-type ES cells, as well as previously generated maps from different organisms, were generally in agreement. They showed the presence of nucleosome-depleted regions (NDRs) upstream of transcription start sites (TSSs) and at transcription termination sites (TTSs) in actively transcribed genes. By contrast, the chemical map revealed generally high nucleosome occupancy spanning the TSS, coding sequence and TTS of actively transcribed genes.

To investigate how nucleosome positioning correlates with RNA polymerase II (Pol II) elongation kinetics, the authors used an available global run-on and sequencing (GRO-seq) data set, from which sites of Pol II accumulation can be inferred. Alignment of Pol II accumulation at promoter-proximal sites with the chemically determined nucleosome positions revealed that occupancy of the +1 position relative to the TSS was positively associated with Pol II pausing.

The rate of transcription can influence co-transcriptional splicing. In contrast to recent studies, which found that nucleosomes have higher occupancy around exon centres, the chemical map revealed that nucleosomes are enriched at exon boundaries. Importantly, at all of the expressed genes (regardless of expression levels), Pol II accumulation correlated with nucleosome occupancy at exon boundaries, which is indicative of Pol II stalling at exon–intron junctions close to the nucleosome centre, where the strongest DNA–histone interactions occur.

It is still unclear whether pluripotency transcription factors can bind to their target sites when the DNA is bound by nucleosomes. The chemical map showed that the pluripotency factors OCT4, SOX2, Nanog and Krüppel-like factor 4 bind to their target sites within nucleosomes and modulate nucleosomes in the flanking regions. This suggests that they function as pioneer factors, which can induce chromatin opening and the formation of NDRs.

This study supports a dynamic function of nucleosome in gene regulation. At both promoter-proximal regions and exon–intron junctions, nucleosomes could function as transient barriers for Pol II progression, thereby regulating the kinetics of transcription elongation and splicing.

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ORIGINAL ARTICLE Voong, L. N. *et al.* Insights into nucleosome organization in mouse embryonic stem cells through chemical mapping. *Cell* **167**, 1555–1570 (2016)
FURTHER READING Jonkers, I. & Lis, J. T. Getting up to speed with transcription elongation by RNA polymerase II. *Nat. Rev. Mol. Cell Biol.* **16**, 167–177 (2015)