

# Transcription from A to H2A.Z

April 21, 2014

GE Zentner

Eukaryotic genomes are packaged into chromatin, comprised of nucleosomes formed by the wrapping of ~147 base pairs (bp) of DNA around octamers of basic histone proteins. Chromatin restricts access to DNA, and so any processes requiring contact with DNA must first contend with chromatin structure. The relationship between chromatin and one such process, gene transcription, has been unclear. In vitro, a single nucleosome presents a major barrier to transcription by RNA polymerase II (Pol II), which transcribes the majority of protein-coding genes. In vivo, Pol II must transcribe at high rates across many nucleosomes in order to achieve expression of thousands of genes, but how this occurs has been unclear. To comprehensively determine the in vivo relationship between nucleosomes and transcription, graduate student Christopher Weber and post-doc Srinivas Ramachandran, in the laboratory of Dr. Steven Henikoff (Basic Sciences Division), developed a new technique that enables precise mapping of Pol II on chromatin. This technique, termed profiling of 3' ends of nascent transcripts (3'NT), maps the last nucleotide added to a transcript by Pol II. "Using 3'NT, we were able to profile total Pol II at base-pair resolution, which allowed us to determine the precise nature of the nucleosomal barrier to Pol II transit," said Weber.

To develop 3'NT, the researchers made use of the remarkable stability of the Pol II-DNA-RNA complex, which resists disruption by nucleases, high salt, and harsh detergents. To obtain Pol II-DNA-RNA complexes, the researchers lysed cells to release cytoplasmic and nuclear proteins and washed isolated chromatin extensively to remove non-Pol II-associated RNA. The remaining material contained nearly all cellular Pol II. Nascent RNAs were then sequenced and their 3' ends were mapped back to the *Drosophila* genome. At the gross level, accumulation of 3'NT signal showed good correlation with nucleosome positions, in agreement with Pol II stalling at nucleosomes. Robust correlation between 3'NT signal and Pol II positions was also observed when determined by a lower-resolution technique, chromatin immunoprecipitation and high-throughput sequencing (ChIP-seq).

The authors noted that the majority of 3'NT signal occurred just upstream of the first nucleosome (+1 nucleosome) that Pol II encounters. Averaging 3'NT signals for thousands of genes, they found that the major site of stalling occurs near the entry site of DNA into the nucleosome. As nucleosomes protect ~147 bp of DNA, they concluded that entry to the +1 nucleosome presented the strongest barrier to Pol II passage.

The researchers hypothesized that the nature of the nucleosomal barrier to Pol II passage might depend on the context of the nucleosome; that is, the +1 nucleosome is flanked by only one nucleosome downstream and by a nucleosome-depleted promoter upstream, while nucleosomes further into gene bodies are flanked by nucleosomes on both sides. Analyzing 3'NT data for the second nucleosome in gene bodies (+2 nucleosome), the authors found that Pol II did stall at the entry site of the +2 nucleosome, similarly to the +1 nucleosome, but with lower magnitude. For nucleosomes further into gene bodies, entry site stalling was also observed, again with lower magnitude than +2 nucleosomes, and there were additional stall positions further into the nucleosome. From this, the authors concluded that the first nucleosome that Pol II encounters is the highest barrier to its passage.

As the histone variant H2A.Z, an alternative form of histone H2A, is highly enriched at the +1 nucleosome position, where the barrier to Pol II is highest, the authors asked if H2A.Z influenced Pol II stalling. Using RNA interference (RNAi), they depleted H2A.Z and found that genes with decreased H2A.Z incorporation at +1, +2, and other gene body nucleosomes displayed increased stalling, indicating that H2A.Z facilitates transcription through nucleosomes. This function may be particularly pertinent during development, as H2A.Z knockout mice die at gastrulation, when complex gene expression patterns are being established in the embryo.

These results provide, at base-pair resolution, fundamental insights into the process of transcription in vivo. "Our results point to a different mechanism of Pol II transit across nucleosomes in vivo compared to existing models, and that both context and composition influence this process," said Weber.

[Weber CM, Ramachandran S, Henikoff S](#). 2014. Nucleosomes are Context-Specific, H2A.Z-Modulated Barriers to RNA Polymerase. *Mol Cell* 53(5):819-830.

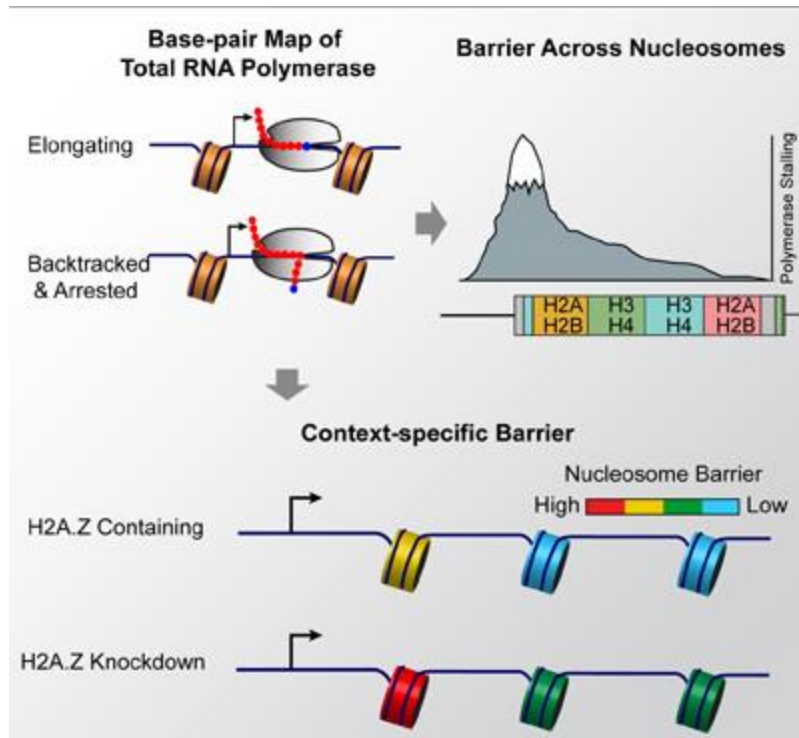


Image provided by Christopher Weber

(Upper left) Schematic illustration of the RNA polymerase II states profiled by 3'NT. The 3' nucleotide of each transcript is colored blue. (Upper right) Schematic of where RNA polymerase II stalls with respect to a nucleosome. The leading edge of the nucleosome, represented as a mountain, is the highest barrier to RNA polymerase II transit. (Bottom) Effect of H2A.Z-containing nucleosomes on RNA polymerase II. Depletion of H2A.Z increases the height of the barrier to RNA polymerase II transit, resulting in increased stalling.