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"Visualizing transcription at nucleotide resolution by nascent transcript sequencing"

A longstanding paradox in cell biology is how the extended strands of DNA can both be neatly packaged into protein structures (nucleosomes) and remain accessible to RNA polymerase. Outside the cell, nucleosomes can entirely halt RNA polymerase. Thus in vivo, the structure is either eliminated or RNA polymerase has the help of other proteins to navigate through these barriers. Moreover, transcription is far more complicated than what was thought just a few years ago both in the intricate use of post-initiation control and the mass production of rapidly degraded transcripts.

To address these complexities, I developed a high precision method to look at the action of RNA polymerase in cells. Here I present a simple and powerful approach (Native Elongating Transcript sequencing, NET-seq) that monitors transcription in the cell at single nucleotide resolution. NET-seq, by providing a quantitative, non-perturbative measure of transcription initiation, elongation and termination at nucleotide resolution, allows for the in-depth investigation of these complexities providing high precision measurements directly comparable to in vitro biophysical studies. I used NET-seq to address two fundamental questions: how do promoters dictate their directionality and what is the impact of nucleosome structure on the elongating RNA polymerase? Among other things, our studies established that nucleosome-induced pausing represents a major barrier to transcriptional elongation in vivo.