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Investigation of RNA Polymerase I Transcription under Force-Free Condition by Single Molecule Technique SULEYMAN UCUNCUOGLU, Emory University, DAVID A. SCHNEIDER, University of Alabama at Birmingham, DAVID DUNLAP, LAURA FINZI, Emory University — RNA Polymerase I (Pol I) conducts more than 60% of all the transcriptional activity in cells and also is responsible for synthesizing the RNA structure of the ribosome in eukaryotic cells. It is evident in many studies that Pol I transcription is affected by tumor suppressors and oncogenes which makes Pol I as a target for the anticancer therapeutics. The mechanistic pathways and kinetics of the Pol I transcription needs to be understood more precisely. Even though previous bulk studies measured the kinetics of the Pol I transcription, the results may hinder the intermediate states such as processivity and pausing during elongation. Here we used the single molecule approach to show that Pol I pauses more than Pol II during elongation step by using a novel single molecule instrument, multiplexed tethered particle motion microscopy (TPM). Our in-house developed TPM equipment is able to concurrently observe hundreds of single molecules. TPM technique has a major advantage to observe pausing under force-free condition unlike other single molecule techniques such as magnetic tweezers and optical tweezers. We also report that the processivity of Pol I is very low where only one out of fifteen transcription event reached the run-off site. We anticipate that our single molecule assays paved the way for observing more sophisticated aspects of Pol I transcription and it's relation with initiation and transcriptional factors.

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