Single-molecule studies of RNA polymerase II transcription

Inwha Baek[1], Grace Rosen[2], Larry J Friedman[2], Yoo Jin Joo[1], Jeff Gelles[2], and Stephen Buratowski[1]

1 Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

2 Department of Biochemistry, Brandeis University, Waltham, MA, 02454, USA

Abstract

Transcription is the fundamental biological process by which RNA transcripts are synthesized from a DNA template. In eukaryotes, RNA polymerase II (Pol II) transcribes messenger RNAs and non-coding RNAs. There has been remarkable progress in our understanding of Pol II transcription. However, the association and dissociation kinetics of transcription factors during Pol II transcription were poorly characterized since the past studies utilized ensemble assays, which can only reveal the time- and population-averaged behavior of molecules. In this study, we implemented multi-wavelength single-molecule fluorescence microscopy to investigate the dynamics of Pol II and transcription factors during activator-dependent transcription reactions. The reactions were performed in a budding yeast nuclear extract, known to faithfully recapitulate activator-dependent Pol II transcription.

Single-molecule imaging revealed several unexpected features of Pol II transcription activation and PIC assembly. Pol II and general transcription factors TFIIF and TFIIE can pre-assemble on enhancer-bound activators before loading into a preinitiation complex (PIC) at the core promoter. More interestingly, multiple Pol II·TFIIF·TFIIE complexes can simultaneously bind a single enhancer, creating a localized cluster. Unlike other factors, TFIIH required the core promoter DNA for its recruitment and only a single TFIIH molecule associated with the DNA template. Our kinetic measurements lead to a new branched model for activator-dependent PIC assembly. Single-molecule measurements also showed a major rate-limiting step for PIC and elongation complex assembly, manifested in a lag before TFIIH and the elongation factor Spt4/5 bindings to DNA. Altogether, this study uncovers the kinetics of Pol II and transcription factors during activator-dependent transcription that have been masked by ensemble techniques and provides mechanistic insight into how activators promotes PIC assembly.