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Single-molecule investigations of transcription with powerful new imaging and computational tools: from DNA-template interactions to gene regulation. Single-molecule imaging and fluorescence spectroscopy have opened a window into the level of control afforded by biological structures and processes. These techniques can be used to gain insight into the mechanisms of macromolecular interactions that occur in the context of the living cell. Single-molecule methods have also been used to characterize the intrinsic dynamics of individual molecules. Such studies of the distribution of intrinsic conformational states within a single molecule (as opposed to ensemble measurements) can reveal how fluctuations within a sample translate to in vivo behavior. Here we review an array of single-molecule studies that have been used to investigate the mechanisms and consequences of DNA-template interactions in transcriptional regulation. These methods include the measurement of polymerase template search dynamics, binding site durations on DNA templates, and protein-DNA binding dynamics. We describe how such single-molecule methods can be used to gain insight into the functions of cellular factors in a cellular context and offer predictions for the behavior and regulation of cellular functions in living cells. 6d1f23a050

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