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Single-molecule and super-resolution imaging of transcription in living bacteria

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ABSTRACT

In vivo single-molecule and super-resolution techniques are transforming our ability to study transcription as it takes place in its native environment in living cells. This review will detail the methods for imaging single molecules in cells, and the data-analysis tools which can be used to extract quantitative information on the spatial organization, mobility, and kinetics of the transcription machinery from these experiments. Furthermore, we will highlight studies which have applied these techniques to shed new light on bacterial transcription.

Contents

Abbreviations: FP, fluorescent protein; PAFP, photoactivatable fluorescent protein; RNAP, RNA polymerase; dSTORM, direct stochastic optical reconstruction microscopy; PALM, photoactivated localization microscopy; sptPALM, single-particle tracking PALM; bp, basepair; SIM, structured illumination microscopy; EMCCD, electron multiplying charge coupled device; FRAP, fluorescence recovery after photobleaching; MSD, mean squared displacement; FROS, fluorescent repressor operator system.

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1. Introduction

Transcription is one of the most fundamental processes necessary for life, being the first step in gene expression and ultimately responsible for how both eukaryotic and prokaryotic cells respond to changes in their environment. In bacteria, unlike eukaryotes, there is only a single type of RNA polymerase (RNAP) responsible for transcription of both coding and non-coding RNA. RNAP is a multi-subunit protein machine made up of a beta and a beta prime subunit, two alpha subunits, and an omega subunit. In order to bind promoters, the RNAP core associates with transcription initiation sigma factors (σ factors) to form the RNA polymerase holoenzyme; in the case of the *Escherichia coli* housekeeping σ factor (σ^{70}), this association forms a 450 kDa holoenzyme [1]. Sigma factors reduce the affinity of RNAP for non-specific DNA while increasing specificity for promoters.

There are ~2000 σ^{70}-specific promoters in *E. coli* [2], each containing a core sequence of ~40 base pairs (bp) in length, with two short sequences approximately −10 and −35 bp upstream of the transcription start site. Taken together, these promoter sequences account for less than 2% of the *E. coli* genome [3]. In order to locate a promoter, an RNAP molecule must therefore discriminate between vast amounts of nonspecific DNA.

After initial binding to the promoter, RNAP opens a bubble in the duplex DNA to form an 'open complex' and begins transcription (Fig. 1) [4,5]. In bacteria, transcription and translation are not segregated, and ribosomes can form on the nascent transcript as soon as the ribosome binding site has emerged from the RNA-exit channel of RNAP. At some point during elongation, the sigma factor usually dissociates and is free to associate with another core enzyme [6]. Finally, RNAP reaches the end of the gene, and the RNA transcript and the core enzyme dissociate from DNA.

At the molecular level, much of our understanding of transcription is based on *in vitro* experiments performed using purified proteins and DNA. The finest level of detail has been achieved through X-ray crystallography, allowing the precise interactions between the bases on the DNA and the amino acid residues on the transcription machinery to be determined. However, the 'snapshots' from crystallography are poorly suited to studying dynamic behavior. To complement structural information from crystallography, *in vitro* single-molecule experiments are becoming increasingly popular tools to study transcription, since they can determine the kinetics of these interactions by directly observing the behavior of individual molecules [7–17]. While *in vitro* single-molecule techniques have been used to great effect in elucidating molecular behavior, care must be taken when inferring the physiological relevance, since these experiments are performed on highly simplified systems and in isolation from the rest of the cellular components.

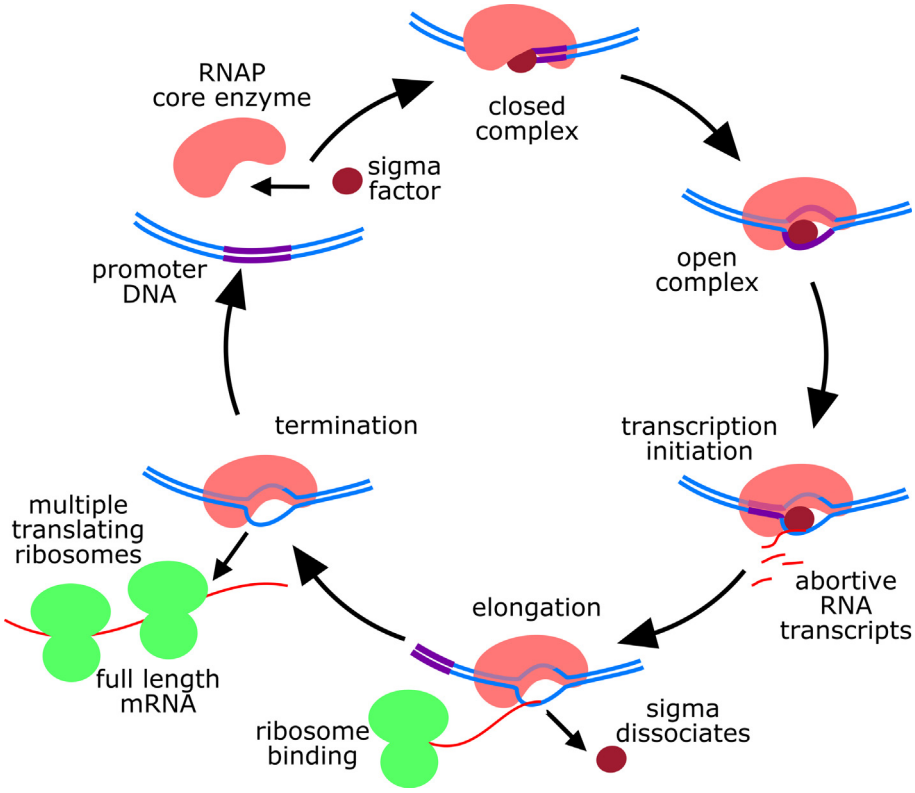

Fig. 1. The transcription cycle. RNAP associates with a sigma factor before binding to a promoter site. After initial binding, the enzyme opens a bubble in the duplex DNA to form an 'open complex'. From here, it can initiate transcription; however, on many promoters, the polymerase makes several attempts to start transcribing, generating short abortive RNAs [14]. Once past the ~10th nucleotide, the RNAP breaks its interactions with promoter DNA and enters into processive synthesis of RNA as an 'elongation complex'. At some point during elongation, the sigma factor usually dissociates from the core enzyme [6]. Finally, RNAP reaches the end of the gene, and the RNA transcript and the core enzyme dissociate from DNA.