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Single-molecule studies of RNAPII elongation

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Abstract

Elongation, the transcriptional phase in which RNA polymerase (RNAP) moves processively along a DNA template, occurs via a fundamental enzymatic mechanism that is thought to be universally conserved among multi-subunit polymerases in all kingdoms of life. Beyond this basic mechanism, a multitude of processes are integrated into transcript elongation, among them fidelity control, gene regulatory interactions involving elongation factors, RNA splicing or processing factors, and regulatory mechanisms associated with chromatin structure. Many kinetic and molecular details of the mechanism of the nucleotide addition cycle and its regulation, however, remain elusive and generate continued interest and even controversy. Recently, single-molecule approaches have emerged as powerful tools for the study of transcription in eukaryotic organisms. Here, we review recent progress and discuss some of the unresolved questions and ongoing debates, while anticipating future developments in the field.

Keywords

Pol II; transcript elongation; single-molecule

1. Introduction

Much of our current understanding of transcription has come from ensemble-based measurements, performed using traditional biochemical approaches in which RNA polymerases and their accessory factors were first isolated, and then their behavior analyzed. These careful measurements have been complemented by structural studies that provide breathtaking ‘still pictures’ of the transcriptional apparatus (Kettenberger et al., 2004; Wang et al., 2009) with spatial resolution down to the atomic-scale. The information from such structures has been integrated to the point that we can now construct ‘movies’ of the nucleotide addition cycle (Brueckner et al., 2009) based on snapshots of RNAPII in different intermediate states. However, despite these successes, our understanding of the detailed structural rearrangements and the kinetics of the steps connecting these states is far from complete.

In particular, every substep of the nucleotide addition cycle is a potential target of an intricate network of gene regulatory pathways, and every nucleotide addition cycle is subject to scrutiny by fidelity control (error correction) mechanisms that ensure faithful transcription of the genetic code. Individual enzymes frequently switch between periods of productive elongation and off-pathway states with regulatory significance, often in an apparently stochastic manner. It is notoriously difficult to study such complex pathways in bulk biochemical experiments, where it is impossible or impractical to resolve rare stochastic

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events, and mixed populations of enzymes in both active and inactive states co-exist. Single-molecule approaches, by contrast, can avoid the complications of ensemble averaging, providing dynamic information about the kinetics of individual enzymes with comparatively high spatial and temporal resolution.

Over the past two decades, with the ability to probe directly the transient, unsynchronized behavior of RNAP molecules, single-molecule techniques have provided fresh insights into the mechanisms of transcript elongation. Broadly speaking, three classes of single-molecule techniques have been applied to the study of transcription: single-molecule fluorescence (e.g., single-molecule Förster resonance energy transfer, or smFRET), atomic force microscopy (AFM), and displacement-based methods that attach tiny particles to polymerases and track their motions, e.g., optical traps, magnetic tweezers, and tethered particle motion (TPM) assays (reviewed in (Greenleaf et al., 2007)). In particular, single-molecule optical trapping assays of transcription have progressed dramatically, tracking the motion of RNA polymerase along DNA with a spatial resolution down to single base-pairs (Abbondanzieri et al., 2005). Past work has largely concentrated on the prokaryotic form of RNAP (Herbert et al., 2008), which is straightforward to initiate on a DNA template from a promoter site, after which elongation can be studied. Only recently has it become practical to circumvent the complex initiation process generally required for eukaryotic RNA polymerases, which involves multiple transcription initiation factors. *In vitro*, functional elongation complexes can be created in the absence of such factors by assembling a scaffold of DNA and RNA around the enzyme that mimics the transcription bubble assembly. Using this approach, high-resolution optical trapping studies have since been employed to study the mechanochemistry of RNAPII (Galburt et al., 2007; Larson et al., 2012), as well as to probe its interactions with factor TFIIS (Galburt et al., 2007), and to study transcription through nucleosome (Hodges et al., 2009). Complementary work using FRET-based approaches has begun to unravel the interactions of RNA polymerase with nucleic acids at nanometer spatial resolution (Andrecka et al., 2008; Andrecka et al., 2009; Chen et al., 2009; Muschielok et al., 2008).

In parallel with these developments, dramatic progress has been made using live-cell imaging approaches, based on the expression in *in vivo* of fluorescent proteins. Single-molecule imaging studies of transcription *in vivo* have become feasible as well, and have led to a picture of gene expression in which many key molecular players (transcription factors, mRNA transcripts, etc.) exist at the level of only a few molecules per cell. As a consequence of the small numbers, stochastic events in transcriptional and translational processes become important in determining cellular fates (Choi et al., 2008). Recently, single-molecule studies *in vivo* have been extended to the study of eukaryotic transcription as well, visualizing the kinetics of the initiation and elongation phases of transcription, revealing heterogeneities in the rates of transcription at different stages of the cell cycle (Larson et al., 2011).

Here, we review recent progress in single-molecule assays developed to study eukaryotic transcriptional elongation, the kinetics and fidelity control of the nucleotide addition cycle, and the interactions of RNAPII with nucleosomes and transcription factors. We end by presenting opportunities and challenges for future single-molecule studies, and discuss their potential synergy with emerging, genome-wide approaches to eukaryotic transcription.

Introduction to high-resolution optical trapping assays of transcription

Optical trapping techniques allow the motions of single RNAP molecules to be monitored at high spatiotemporal resolution as these transcribe a DNA template. In particular, the “dumbbell” optical trapping assay (Figure 1A) pioneered by the Block group has been able to achieve angstrom-level resolution, which is sufficient to resolve motions across single

base pairs, allowing transcription to be investigated at the level of each nucleotide addition cycle (NAC) (Abbondanzieri et al., 2005). Representative single-molecule elongation records of template position *versus* time (Figure 1C) reveal a number of features. The sloped segments of such records indicate periods of active movement along the template, and supply the enzyme's velocity during active elongation. Transient transcriptional pauses are evident as horizontal segments of various durations. Single-molecule experiments thus allow one to distinguish in a straightforward manner between periods of active elongation and pausing. Optical traps can also be used to apply carefully controlled loads (up to ~40 pN) along the DNA (or RNA) that either assist or hinder the translocation of RNAP. Because both active elongation and certain mechanisms that can lead to pausing (e.g., backtracking) involve the movement of RNAP along DNA, measuring the force dependence of such processes can provide insights into candidate mechanisms. These characteristics have enabled kinetic and mechanistic details of transcriptional elongation by bacterial RNAP and its modulation by transcription factors (such as Gre factors, NusA and NusG) to be studied (Abbondanzieri et al., 2005; Dalal et al., 2006; Herbert et al., 2010; Neuman et al., 2003; Shaevitz et al., 2003; Zhou et al., 2011).

Single-molecule studies of eukaryotic transcription using optical traps (and other single-molecule techniques) did not really emerge until 2007, more than a decade after the first single-molecule and optical trapping studies of prokaryotic transcription (Schafer et al., 1991; Wang et al., 1998; Yin et al., 1995). The source of this delay is largely attributable to the complex initiation process of eukaryotic RNA polymerases. In contrast to bacterial RNAP, which requires a single σ -cofactor to initiate transcription, initiation by eukaryotic RNAPII requires a minimal set of five general transcription factors (TFIIB, TFIID, TFIIE, TFIIIF and TFIIH) (Conaway and Conaway, 1997). Purifying these factors is a non-trivial task, and combining all of them with RNAPII to yield functional pre-initiation complexes with high efficiency *in vitro* has proved to be exceedingly difficult. In 2003, Kashlev and coworkers successfully developed a 'scaffolding' approach that circumvents the normal initiation process, and were thereby able to engineer functional elongation complexes (EC) in the absence of any general transcription factors, using only yeast RNAPII and synthetic DNA and RNA oligonucleotides (Komissarova et al., 2003). The sequential, stepwise assembly of the EC is the key to this assay (Figure 1B). In brief, a DNA-RNA hybrid (~9 bp) is first produced by pairing a relatively long (>30 bp) template DNA strand with a short RNA oligomer (~10 bp). Next, the polymerase is introduced, and it binds to the hybrid region. Finally, a DNA oligomer that will serve as the non-template strand is added, forming a transcription bubble that is competent for active elongation. By adapting this approach to an optical trapping assay, Bustamante and coworkers were able to monitor transcriptional elongation by RNAPII at the single-molecule level (Galburt et al., 2007). In that study, to eliminate potential inactive ECs on the trap, they also incorporated a restriction site that should be protected by active ECs after adding the initial subset of NTPs. Inactive ECs that failed to protect this site, therefore, would be digested away and no longer able to form tethers. Following similar stepwise assembly approaches, Landick and coworkers developed an assembly method for engineering EC for calf-thymus RNAPII (Palangat et al., 2012), and Block and coworkers have recently carried out single-molecule studies of transcriptional elongation in both yeast and calf-thymus RNAPII (Larson et al., 2012).

2. Kinetics and chemomechanics of eukaryotic transcriptional elongation

2.1 Distinguishing candidate translocation models for the NAC

Active elongation by RNAPII (and also by bacterial RNAP) proceeds in a repetitive manner, with each RNA nucleotide in sequence. The NAC consists of a set of reaction steps that comprise, at a minimum: translocation of the elongation complex (EC) from its pre-translocated to the post-translocated register, substrate nucleoside triphosphate (NTP)

binding, catalysis (condensation of the NTP into RNA), and pyrophosphate (PPi) release. Understanding the kinetic details and molecular mechanisms of these steps is crucial for an understanding the overall mechanism of elongation and its regulation by the microenvironment (e.g., NTP and PPi concentrations) and by transcription factors. Previous single-molecule and ensemble experiments suggest that transcription factors affect active elongation and pausing by modulating the rate of the translocation step in bacterial RNAP (Bar-Nahum et al., 2005; Zhou et al., 2011). Although the NAC has been studied by a variety of biochemical, structural, genetic, and single-molecule approaches, details of the kinetics and molecular mechanisms remain a source of controversy. This is, at least in part, because translocation, the key step in the NAC, is particularly poorly characterized, and its placement within the NAC is uncertain.

At least two classes of translocation model currently exist in the field. The first class consists of “power-stroke” models, which were originally inspired by structural and biochemical studies of the bacteriophage T7 RNAP, a single-subunit polymerase (Yin and Steitz, 2004). In such models, translocation is produced by a concerted conformational change (power stroke) in the enzyme that is tightly coupled to NTP hydrolysis and PPi release. However, results from both crystal structure analysis and biochemical experiments appeared to disfavor this model, at least for multi-subunit polymerases (Kireeva et al., 2010; Komissarova and Kashlev, 1997; Wang et al., 2006). The strongest evidence disfavoring this class of model arguably comes from the kinetics measured in high-resolution single-molecule experiments (Abbondanzieri et al., 2005). The second class of model consists of “Brownian ratchet-type” mechanisms (Figure 2), in which thermal fluctuations between the pre- and post-translocated states of RNAP get mechanically rectified by other kinetic steps that release energy (e.g., NTP binding, catalysis, pyrophosphate release, etc.), leading to net, unidirectional enzyme movement. Although Brownian ratchets are currently favored over power stroke models, many open questions remain. In particular, the placement of translocation step among the other kinetic transitions, and the pathway for nucleoside triphosphate entry into the active site, are currently in contention. Some experimental (Bai et al., 2007; Bar-Nahum et al., 2005; Komissarova and Kashlev, 1997) and theoretical (Bai et al., 2004; Guajardo and Sousa, 1997) evidence suggests that NTP binding is responsible for rectifying the post-translocated state of RNA polymerase. In this version of the ratchet model, NTP can only bind to RNAP in the post-translocated state (Figure 2A). This is consistent with most structural data suggesting that the RNAP secondary pore is the only (or dominant) route for the entry of NTP, and the active “A” site may only be available for incoming NTP substrate in the post-translocated EC (Cramer et al., 2001; Kettenberger et al., 2004; Vassilyev et al., 2007b; Westover et al., 2004a). A somewhat different model, inspired by results from pre-steady-state analyses, suggests that NTP binding may precede, and possibly stimulate, translocation both in *E.coli* RNAP and human RNAPII (Foster et al., 2001; Kennedy and Erie, 2011; Kireeva et al., 2010; Nedialkov et al., 2003). An important implication of the latter model is that there exists some secondary site allowing NTP to bind in the pre-translocated state of the EC. In this case, it is proposed that NTPs enter through the downstream DNA-binding channel (main channel), bind to template DNA downstream of the catalytic site, and then transfer quickly to the active “A” site. This proposed pathway is potentially compatible with certain biochemical experiments and structural data (Burton et al., 2005; Holmes and Erie, 2003; Westover et al., 2004a, b). However, direct experimental proof for such a secondary NTP binding site is lacking, and neither a kinetic nor structural model that explains how an NTP might be able to load via the main channel currently exists. Finally, because kinetic studies demonstrate that the slow rate of PPi release is stimulated by the incoming complementary NTP (Johnson et al., 2008), the placement of the PPi release step with respect to translocation and NTP binding steps is also uncertain.

To study the NAC in RNAPII and distinguish among the candidate biochemical pathways for translocation (Figure 2), Larson and Zhou (Larson et al., 2012) carried out single-molecule optical trapping experiments and fit the resulting force-velocity curves to four different Brownian ratchet models. In this study, the pause-free velocities were collected under external loads that either assisted or hindered the translocation of yeast RNAPII (wild-type, WT) and two of its trigger-loop (TL) mutants. These experiments were carried out at both saturating and subsaturating NTP levels to resolve catalysis and NTP binding. Because different translocation models predict distinct force-velocity (F - v) relationships at different NTP concentrations, global fits to the force-velocity data could differentiate different models. The best-fit model (Figure 2C) placed the translocation step at the start of the NAC, prior to catalysis and PPi release, with the incoming NTP able to bind RNAPII in either its pre- or post-translocated state. In particular, the F - v data did not fit well to a model where NTP binding is required before forward translocation (Figure 2B), nor did they support a model where the NTP bound exclusively to the post-translocated state (Figure 2A). Taken together with structural data (Cramer et al., 2001; Kettenberger et al., 2004; Vassylyev et al., 2007b; Westover et al., 2004a) indicating that an NTP can only bind the active “A” site in the post-translocated state (Figure 3), the finding that NTP is able to bind both RNAPII (Larson et al., 2012) and bacterial RNAP (Abbondanzieri et al., 2005) in either the pre- or post-translocated state strongly suggests that a secondary NTP binding site likely exists for multi-subunit RNAPs. Moreover, the fact that the global data failed to fit a model where PPi release occurs in either the pre- or post-translocated states (Figure 2D) suggests that the translocation event likely takes place prior to PPi release, although some more complicated kinetic scheme cannot be formally ruled out.

Another noteworthy result from this study is that the translocation equilibrium for yeast RNAPII seems much more biased towards the post-translocated state than that of bacterial RNAP. This implies that the translocation step is comparatively less rate-determining in RNAPII, a mechanochemical characteristic property that may facilitate overcoming nucleosomal barriers by the eukaryotic enzyme. The authors also noted a weak effect of force on the pause-free velocity for WT RNAPII, a dependence that had not been previously reported (Galburt et al., 2007).

2.2 Stall force

The stall force is defined as a (hindering) load sufficient to halt enzyme translocation reversibly. Whereas bacterial RNAP is able to transcribe against quite large hindering loads (~25 pN) (Wang et al., 1998; Yin et al., 1995), a comparatively small force of ~7.5 pN seems to prevent yeast RNAPII from actively elongating (Galburt et al., 2007), (Larson et al., 2012). Interestingly, Galburt et al reported that the presence of TFIIS, a transcription factor that rescues backtracked TECs by activating cleavage of the backtracked RNA and restoring a new 3' end to the active site, permitted about a quarter of RNAPII molecules to transcribe against somewhat greater hindering forces, up to ~17 pN.

As discussed earlier, the F - v relationship of RNAPII implies that it is more biased towards its post-translocated state during active elongation compared to bacterial RNAP. Combined with the observation of a comparatively low stall force, as well as the effect of TFIIS, these data suggest that forces which inactivate elongation may be inducing the EC to enter off-pathway, backtracked states, as well as inhibiting its ability to recover from such backtracked states before becoming irreversibly arrested. We may speculate that for RNAPII, it is mechanically easier to enter off-pathway states, thereby providing a potential mechanism for regulation by different elongation or chromatin remodeling factors.

2.3 Transcriptional pausing kinetics

Transcriptional pausing (Figure 3) plays a critical regulatory role in gene regulation. Pausing modulates the overall elongation rate and therefore provides a mechanism for the modulation of cellular RNA levels. In all domains of life, pausing is known to be modulated by transcription factors. In bacteria, pausing also plays critical roles in synchronizing transcription with translation (Landick et al., 1985), in facilitating the cotranscriptional folding of nascent RNA (Pan and Sosnick, 2006; Wickiser et al., 2005), and in regulating intrinsic and Rho-dependent termination (Gusarov and Nudler, 1999; Kassavetis and Chamberlin, 1981; Richardson, 2002). In eukaryotes, the potential regulatory role of pausing has not been very well characterized and awaits elucidation by future experiments. Nonetheless, recent studies suggest that pausing is involved in a multitude of transcriptional events and may play a role in human disease (Zhou et al., 2012). Pausing plays a critical role in promoter-proximal regulation (Nechaev et al., 2010; Rahl et al., 2010), phosphorylation of the C-terminal domain (Muse et al., 2007), Tat-mediated HIV-1 transcription (Palangat et al., 1998), the formation of so-called “super elongation complexes” (Lin et al., 2011), and a variety of elongation-coupled events, including cotranscriptional splicing (de la Mata et al., 2003), cleavage and polyadenylation (Glover-Cutter et al., 2008; Yonaha and Proudfoot, 1999), and termination (Plant et al., 2005).

Single-molecule studies of eukaryotic RNAPII by Bustamante and coworkers concluded that all pauses are likely to have been generated by enzyme backtracking (Galbur et al., 2007). However, this conclusion must be considered controversial. Owing to limitations in their spatiotemporal resolution, no backtracks of three or fewer base pairs were actually observed. Unfortunately, the vast majority of all pauses falls into this category. Instead, backtracking was inferred indirectly based on a model, by fitting the pause lifetime distribution to a $t^{-3/2}$ power law (for long times). This power-law relationship is the one expected for the first-passage time of a mechanically backtracked enzyme to diffuse, on a random walk, back to its initial position and resume elongation. Inspired by these studies, a subsequent theoretical study modeled backtracking as a force-biased random walk of the enzyme over a lattice of upstream template positions. It concluded that all pauses in yeast RNAPII and bacterial RNAP could be a consequence of backtracking (Depken et al., 2009). This “backtracking-only” model for transcriptional pausing is attractive in its simplicity, but it is questionable whether such a simplistic mechanism can satisfactorily explain the large body of existing biochemical and single-molecule data on transcriptional pausing, which has led others to conclude that there are multiple mechanisms for pausing, and that certain types of regulatory pause exist which are not associated with backtracking.

In particular, Block and coworkers have identified and characterized, in *E. coli* RNAP, a class of pauses termed “ubiquitous pauses”. Studies suggest that these ubiquitous pauses are frequent, brief, sequence-dependent, and not backtracked (Herbert et al., 2006; Neuman et al., 2003; Zhou et al., 2011). More specifically, the force dependences of the densities and lifetimes of these ubiquitous pauses are incompatible with the backtracking-only model in its present form. Ubiquitous pauses are thought to correspond to ‘elemental’ pauses that branch off the main elongation pathway due to an active-site rearrangement, i.e., fraying of the 3′ end of RNA (Kireeva and Kashlev, 2009; Touloukhonov et al., 2007). Enzymes in this state can subsequently transition to long-lived pauses by either backtracking of the RNAP or hairpin formation in the nascent RNA. Because multi-subunit RNAPs are remarkably conserved in evolution, it seems plausible that ubiquitous pauses are also generated during transcription by RNAPII, but this is clearly a question that will need to be addressed by future, single-molecule research. High-resolution studies are uniquely suited to explore the various pausing mechanisms by RNAPII, utilizing a range of loads, conditions, and DNA sequences.

3. RNAPII elongation dynamics and fidelity are governed by essential subdomains

Two important RNAPII subdomains adjacent to the active site, termed the bridge helix (BH) and trigger loop (TL), have received considerable attention over the past few years. These regions are evolutionarily conserved in a variety of multi-subunit RNA polymerases, including eukaryotic RNAP I, II, III, and bacterial and archaeal RNAP. Structural, genetic, and biochemical studies suggest that the BH and TL subdomains play a fundamental role in the transcription process, and alterations of the TL have important consequences for both the elongation velocity and transcriptional fidelity of the enzyme (Bar-Nahum et al., 2005; Kaplan et al., 2008; Kireeva et al., 2008; Vassilyev et al., 2007b; Wang et al., 2006). Despite much work, fundamental questions about mechanism remain. The overall elongation rate is determined by the active (i.e., pause-free) elongation rate, set by the kinetics of the NAC, and also by the kinetics of any pausing. An understanding of the ways in which the BH and TL modulate the elongation rate, therefore, requires a detailed understanding of the individual steps within the NAC, as well as their interplay with any off-pathway events, such as pausing.

Roles for the BH and TL in transcriptional fidelity have also been proposed. Fidelity is achieved via a multi-step process. (1) A baseline level of fidelity is conferred by the preferential catalytic incorporation of the correct NTP *vs.* non-cognate NTPs, or NTP-analogs (Yuzenkova et al., 2010), presumably based on base-pairing interaction energies. The binding of the cognate NTP is estimated to be favored by ~100-fold over binding of non-cognate NTPs, and may also be kinetically favored by active site conformational changes centered on the TL (Kaplan et al., 2008; Kireeva et al., 2008; Vassilyev et al., 2007b; Wang et al., 2006). (2) Following any misincorporation event, the rate of next-nucleotide incorporation is slowed dramatically, allowing for error correction via RNAPII-mediated transcript cleavage, or by TFIIIS-enhanced transcript cleavage. (3) Additional fidelity may arise from templated NTP binding to DNA at noncatalytic sites in the downstream DNA entry channel (Gong et al., 2005).

To investigate the interplay between elongation rate and transcriptional fidelity, one recent single-molecule study examined the kinetics of the NAC in two RNAPII mutants, carrying either the E1103G or H1085A/E1103G mutations in the trigger-loop region (Figure 4) (Larson et al., 2012). By comparing the force-velocity relationships of these TL mutants at different NTP concentrations with that of the WT, they found evidence that the RNAPII TL serves as a central regulatory element that affects each of the three main phases of elongation: substrate selection, translocation, and catalysis. The authors also studied fidelity control by examining pausing kinetics, which play a role in proofreading (Erie et al., 1993; Shaevitz et al., 2003). For example, following misincorporation of certain bases, such as the GTP analog ITP, bacterial RNAP is known to backtrack along the template and enter into a relatively long-lived, paused state. The backtracked polymerase can be rescued from this state by transcription factors (such as Gre factors) that activate cleavage of the most recently incorporated bases (Erie et al., 1993; Izban and Luse, 1992; Shaevitz et al., 2003). Under conditions that promote base misincorporation, a comparison of the pausing kinetics for WT enzyme and the TL mutant (E1103G) suggests that the trigger loop is involved in fidelity in both substrate selection and mismatch recognition (Larson et al., 2012).

4. RNAPII elongation in the presence of nucleosomes

Compaction of genomic DNA into chromatin is a unique feature of eukaryotic cells. Nucleosomes, the elemental packing units of chromatin, have recently emerged as central players in eukaryotic gene regulation. A number of single-molecule experiments have

recently been carried out to understand how RNA polymerase elongates in the presence of nucleosomes.

The first such study was performed by Bustamante and coworkers using an optical trapping assay that followed the motion of individual RNAPII molecules as these transcribed DNA templates bound to a single nucleosome (Hodges et al., 2009). The presence of the nucleosome significantly impeded elongation, causing a sizeable fraction of RNAPII molecules to arrest at the nucleosome under conditions of low ionic strength. Increasing the ionic strength led to reduced histone-DNA interaction and significantly reduced the frequency of nucleosomal arrest, consistent with previous ensemble observations (Bondarenko et al., 2006). For the RNAPII molecules encountering the nucleosome at high ionic strength, dramatically increased pause densities and lifetimes were observed, with the strongest effect occurring before RNAPII reached the dyad axis of the nucleosome. This result is in agreement with a recent *in vivo* study of transcriptional pausing using nascent transcript sequencing (Churchman and Weissman, 2011) and is consistent with previous *in vitro* ensemble experiments (Kireeva et al., 2005). Besides enhanced pausing, a significant reduction in the pause-free elongation rate was reported. Based on their data, Hodges et al proposed a brownian ratchet-type model in which RNAPII ratchets forward as nucleosomes unwrap locally as a consequence of rapid thermal fluctuations (Hodges et al., 2009). This model disfavors the notion that polymerases actively separate histones from the DNA.

Although a single nucleosome may pose a significant barrier for transcription *in vitro*, the elongation rate in the presence of nucleosomes can nonetheless be fast *in vivo* (Darzacq et al., 2007). Nucleosomal barriers may be decreased, and elongation promoted, by transcription factors that serve to rescue a paused or arrested complex (e.g., TFIIS), by various histone modifications (Brown et al., 2000), and by chromatin remodeling factors (Belotserkovskaya et al., 2003; Schwabish and Struhl, 2007). These contrasting mechanisms are likely to be a focus of future single-molecule experiments. Cooperative effects arising from multiple RNA polymerases, acting in concert, may also promote efficient nucleosome traversal, and these have been explored by ensemble experiments (Epshtein and Nudler, 2003; Lee et al., 2004). Recently, a new single-molecule assay has been developed that could also be used for studying transcription of multiple polymerases through a nucleosome (Jin et al., 2010). In their study, Jin and coworkers developed a DNA-unzipping technique that uses optical traps to probe the location of RNAP(s) on a DNA template containing a single nucleosome. Since bacterial RNAP and RNAPII transcribe through nucleosomes using similar mechanisms *in vitro* (Walter et al., 2003), *E. coli* polymerase was used as a model system. The authors found that a single RNAP enzyme backtracks by ~10–15 bp after encountering a nucleosome. However, when a second polymerase was present upstream, a significantly reduced backtracking distance and a five-fold increase in the elongation rate were observed for the leading RNAP, suggesting that the latter may have been “pushed” by elongation of its trailing partner.

A number of studies have examined how transcription affects the nucleosomes that are bound to a DNA template. Previous *in vitro* work on SP6 RNAP and RNAPIII suggests that a transient DNA loop may allow RNAPII to transfer histones to upstream DNA during transcription (Studitsky et al., 1994; Studitsky et al., 1997). *In vivo* and *in vitro* studies of RNAPII suggest that moderate transcription could lead to partial loss of a histone complex (i.e., loss of H2A/H2B dimers), with the remainder of the complex remaining bound to the DNA. Intense transcription by multiple, closely-spaced RNAPII complexes may even induce complete histone eviction (Kulaeva et al., 2010; Thiriet and Hayes, 2005). To test these models of histone transfer during transcription, Bustamante and coworkers monitored the presence of nucleosomes by applying tension between RNAPII and the upstream DNA (Hodges et al., 2009). When transcription was monitored under 3–5 pN of load, which is a

baseline force sufficient to prevent the formation of any DNA loops, subsequent force-extension curves showed no evidence of any histones remaining bound to the DNA template following transcription. However, when transcription was allowed to proceed in the absence of tension, a significant fraction of tethers displayed nucleosome unbinding transitions in their force-extension curves, which may be explained by the existence of histone transfer. To visualize directly the histone transfer, and understand the dynamics of this process, Bustamante and coworkers used AFM to take snapshots of individual TECs during transcription through a nucleosome (Bintu et al., 2011). In that study, the authors found supporting evidence for the formation of DNA loops during histone transfer, and concluded that a small population of nucleosomes moved upstream following transcription (by ~72 bp), while the majority remained at the original location. In addition, the authors found evidence that nucleosomes may form hexamers or octamers upon transcription, depending upon the transcription elongation rate.

5. Position and dynamics of nucleic acids in the TEC

Understanding the architecture of the EC, including the position, structure and dynamics of the various nucleic acids that interact with RNAP (template DNA, non-template DNA and nascent RNA), is critical to an understanding of the mechanism for transcription elongation. Although remarkable progress has been made in obtaining structures for RNAPII (Bushnell et al., 2004; Cramer et al., 2001) and for the EC (Gnatt et al., 2001; Kettenberger et al., 2004; Westover et al., 2004a, b), a complete picture is still lacking.

One vital piece of information that has not been resolved by crystallography is the path by which the nascent RNA exits from polymerase. Two grooves on either side of the “dock” domain of RNAPII were previously proposed to accommodate the exiting RNA: one groove tracks around the base of the “clamp” domain of RNAPII toward subcomplex Rpb4/7, whereas the other goes through Rpb3 and Rpb11 toward Rpb8. Single-molecule FRET experiments are able to monitor small distances (1–10 nm) and thereby detect conformational rearrangements in real time. Two such experiments were recently conducted by independent research groups, using slightly different labeling methods, to distinguish the two RNA exit pathways (Andrecka et al., 2008; Chen et al., 2009). Both sets of results support a model in which the nascent RNA exits toward Rpb4/7, consistent with the path identified previously for the bacterial elongation complex (Vassylyev et al., 2007a). Because the “B finger” of TFIIB has been shown to reach the active center of RNAPII through the same groove (Bushnell et al., 2004), these findings support the notion that the transition from abortive initiation to elongation is largely determined by a steric clash between a growing RNA chain and TFIIB. Moreover, these studies suggest that the 5′ end of RNA can be quite flexible and dynamic when located further away from the active site. For example, the 5′ end of 26-nt RNA could either reside on the “dock domain” (Andrecka et al., 2008) or contact Rpb7 (Chen et al., 2009), as has been suggested by a previous cross-linking study (Ujvari and Luse, 2006).

To ascertain the position and dynamics of mobile elements within the EC (and other macromolecular complexes), Michaelis and coworkers developed a novel single-molecule technique that they call the “Nano Positioning System” (NPS) (Muschielok et al., 2008). In this method, single-molecule FRET efficiencies are measured between an “antenna” dye molecule, labeled to a flexible domain, and several “satellite” dye molecules, attached to known (stable) positions. By applying Bayesian parameter estimation, a statistical method, the most probable coordinates of the antenna, as well as its 3D probability distribution, can be computed. The authors applied this method to re-examine the position of exiting RNA, and confirmed that the 5′ end of RNA transcripts that are 26 and 29 nt long reside on, or close to, the “dock” domain. Another useful application of NPS has been to determine the

position of upstream template and non-template DNA in the EC (Andrecka et al., 2009). The authors showed that the template and non-template strands separate from one another at the +2 position, and that the upstream DNA forms an approximate right angle to the downstream DNA, between the protrusion and clamp of the polymerase. These data match previous studies of the bacterial elongation complex.

6. Outlook

Single-molecule studies of eukaryotic transcription are still in their infancy, and have only begun to touch on the complexities of eukaryotic transcription. Generally speaking, there is a continuing demand for single-molecule studies of eukaryotic systems. A large number of major biochemical activities in eukaryotes are executed under the control of ‘combinatorial’ mechanisms and orchestrated by the actions of multiple proteins. Many of these processes are characterized by a significant degree of stochasticity, by a lack of synchronization between different instances of the same process, and by significant heterogeneity at the molecular level, all of which are difficult to study using conventional, ensemble-based approaches. Thus, eukaryotic transcription may serve as a benchmark system for the development of single-molecule techniques that will complement bulk biochemical and genetic methods, and should be of general use in many future endeavors. In the years to come, we anticipate further progress on multiple fronts, some of which are described below.

Emerging single-molecule techniques which integrate multiple modalities or signals are becoming increasingly capable of studying the actions of enzyme complexes. Notable examples include multi-color fluorescence co-localization techniques (Friedman et al., 2006), which have been applied to study the ordered and dynamic assembly of spliceosomes at the single-molecule level (Hoskins et al.). Other techniques emerging in their own right are combined force-fluorescence spectroscopy (Comstock et al., 2011; Lang et al., 2004) and methods for simultaneous optical trapping and torque spectroscopy (Gutierrez-Medina et al., 2010; La Porta and Wang, 2004). In parallel with these developments in instrumentation, continued advances in the identification, purification and characterization of growing numbers of components involved in transcription, and the *in vitro* reconstitution of more parts of the transcription machinery, are anticipated. The confluence of these advances should enable a wide range of future studies. Among these are: the assembly of the RNA splicing apparatus and other RNA processing factors; the synergistic activities of RNAPII and chromatin remodelers in overcoming nucleosome barriers; the combinatorial control of the phosphorylation state of the CTD of RNAPII’s largest subunit by a variety of kinases and phosphatases; and the ever-changing composition of the EC as the transcription cycle progresses.

One application of combined force-fluorescence techniques may be to study how elongation can be modulated by transcription factors that either transiently or permanently bind to the EC. By labeling transcription factors with fluorophores, and following transcription using single-molecule optical trapping techniques, it may be possible to identify how the binding and dissociation of such factors influences the kinetics of the nucleotide addition cycle. In addition to TFIIS, additional general elongation factors, such as TFIIF (Ujvari et al., 2011; Zawel et al., 1995; Zhang and Burton, 2004), Spt4/5 (Martinez-Rucobo et al., 2011), Elongin, and ELL, await study using single-molecule approaches. Moreover, another specific class of factors is now emerging, exemplified by CTCF (Shukla et al., 2011) and DBIRD (Close et al., 2012), which appear to exert regulatory roles by influencing the elongation of RNAPII at specific locations, such as AT-rich exon/intron junctions.

One major distinguishing feature between bacterial and eukaryotic transcription is the range of processes and events to which it is directly coupled. In bacteria, mRNA gets translated

cotranscriptionally, with transcription and translation being directly coupled (Burmann et al., 2010; Proshkin et al., 2010). In eukaryotes, by contrast, the nascent pre-mRNA is extensively processed and assembled into a messenger ribonucleoprotein (mRNP) particle targeted for nuclear export and cytoplasmic localization. RNA processing events that have been shown to be coupled to transcription in eukaryotes include capping of the 5' end, splicing, polyadenylation of the 3' end, and various mRNA surveillance processes. Such processes may well be amenable to study using the 'RNA pulling' type of optical trapping assays (Dalal et al., 2006; Greenleaf et al., 2008).

Another potentially fruitful avenue amenable to single-molecule experimentation is promoter-proximal pausing, which recently emerged as a widespread transcriptional regulatory strategy, for example, in both fruit fly (Nechaev et al., 2010) and human cells (Min et al., 2011), although it does not appear to be prevalent in yeast. In this process, RNAPII gets transcriptionally stalled immediately following initiation and the transcription of a ~20–50 nt transcript. Promoter-proximal pausing is thought to be controlled by the negative elongation factors NELF and DSIF, and relieved by P-TEFb (Peterlin and Price, 2006). However, the mechanisms by which these factors act, the roles of any pre-initiation complex components that are left behind at the promoter, and the role of the first nucleosome downstream of the promoter region (+1 nucleosome), are presently unknown. Given that promoter-proximal pausing occurs after the transition to an elongation-competent ternary complex, it should be possible to reconstitute this step in single-molecule experiments based on the bubble-scaffold initiation method (Kireeva et al., 2003).

Studying the interactions of transcription machinery with nucleosomes and epigenetic marks is also expected to be a focus of future single-molecule work. Important mechanistic questions in this field have remained unanswered. For example, how do chromatin remodeling factors (such as SWI/SNF, RSC, ISWI, CHD, FACT) (Clapier and Cairns, 2009) facilitate and regulate chromatin transcription? How do they control the disassembly of nucleosomes ahead of RNAPII, as well as the reassembly and repositioning of histones in the wake of transcription? What are the roles of histone variants and posttranslational/epigenetic histone modifications on transcript elongation? Importantly, both nucleosome traversal and chromatin remodeling likely involve DNA un- and re-winding events. Assays based on the recently-developed 'optical torque wrench' thus could provide a unique way to probe such events with high sensitivity.

On the technological side, further improvements in the spatiotemporal resolution of single-molecule experiments can be expected, and we anticipate that assays capable of following transcript elongation on sequence-resolved templates, or with base-pair resolution, will find more widespread use. To align single-molecule elongation records at the base-pair level, past experiments on bacterial RNAP have relied upon the use of periodic templates (tandem repeats) with known pause signals imbedded (e.g., the *his* and *ops* pauses (Herbert et al., 2006)), but such experiments have yet to be carried out with RNAPII at the single-molecule level. High-resolution assays for RNAPII should open up new possibilities for studying the kinetics of the nucleotide addition cycle. Similar experiments will undoubtedly shed more light on the question of how fidelity is achieved during elongation. Additional progress in unraveling the intricacies of the RNAPII enzyme mechanism is likely to come from experiments exploring the properties of RNAP II mutants (Larson et al., 2012).

Synergistic progress may come by interfacing these single-molecule techniques with recent developments in other areas of genetics, biochemistry and biophysics. The last few years have seen the emergence of new genome-wide sequencing methods that can visualize, with a resolution that now reaches the base-pair level, the context in which transcription occurs. Notable results here include high-resolution, genome-wide maps of nucleosome positioning

and occupancy (Gilchrist et al., 2010; Jiang and Pugh, 2009), base-pair resolved information on the positions of nucleic acid binding proteins (Rhee and Pugh, 2011), the architecture of eukaryotic pre-initiation complexes (Rhee and Pugh, 2012), and the position of the active site of RNA polymerase during transcript elongation (Churchman and Weissman, 2011). So far, however, these methods have not admitted to dynamic studies, because they require an averaging of components over many cells. Unique opportunities may therefore exist to study eukaryotic transcription machinery by correlating and juxtaposing findings from genome-wide *in vivo* studies with single-molecule *in vitro* experiments. As an example, Net-seq has begun to unravel the determinants of sequence-dependent RNAPII pausing, backtracking and cleavage by factor TFIIS *in vivo* (Churchman and Weissman, 2011), providing information that can, in principle, be complemented by follow-up optical trapping studies carries out *in vitro*.

A similar potential for synergies exists between single-molecule studies carried out *in vitro* and single-molecule, live-cell imaging work on eukaryotic transcription (Larson et al., 2011). The two approaches yield complementary information. Live-cell experiments can provide the full intracellular context of transcription and its regulation, capitalize on the power of genetic manipulations, and obviate any need for protein purification. So far, however (and despite dramatic improvements in recent years), these experiments lack the resolution of *in vitro* experiments. Significant future efforts will undoubtedly be aimed at bridging this gap from both sides: increasing the resolution and sensitivity of *in vivo* methods where possible, and supplying *in vitro* experiments with more of the components that comprise the cellular context of transcription, at ever-greater levels of assay complexity. Clearly, these are exciting times for single-molecule studies of eukaryotic transcription!

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References

- Abbondanzieri EA, Greenleaf WJ, Shaevitz JW, Landick R, Block SM. Direct observation of base-pair stepping by RNA polymerase. *Nature*. 2005; 438:460–465. [PubMed: 16284617]
- Andrecka J, Lewis R, Bruckner F, Lehmann E, Cramer P, Michaelis J. Single-molecule tracking of mRNA exiting from RNA polymerase II. *Proc Natl Acad Sci USA*. 2008; 105:135–140. [PubMed: 18162559]
- Andrecka J, Treutlein B, Arcusa MA, Muschielok A, Lewis R, Cheung AC, Cramer P, Michaelis J. Nano positioning system reveals the course of upstream and nontemplate DNA within the RNA polymerase II elongation complex. *Nucleic Acids Res*. 2009; 37:5803–5809. [PubMed: 19620213]
- Bai L, Fulbright RM, Wang MD. Mechanochemical kinetics of transcription elongation. *Phys Rev Lett*. 2007; 98:068103. [PubMed: 17358986]
- Bai L, Shundrovsky A, Wang MD. Sequence-dependent kinetic model for transcription elongation by RNA polymerase. *J Mol Biol*. 2004; 344:335–349. [PubMed: 15522289]
- Bar-Nahum G, Epshtein V, Ruckenstein AE, Rafikov R, Mustaev A, Nudler E. A ratchet mechanism of transcription elongation and its control. *Cell*. 2005; 120:183–193. [PubMed: 15680325]
- Belotserkovskaya R, Oh S, Bondarenko VA, Orphanides G, Studitsky VM, Reinberg D. FACT facilitates transcription-dependent nucleosome alteration. *Science*. 2003; 301:1090–1093. [PubMed: 12934006]
- Bintu L, Kopaczynska M, Hodges C, Lubkowska L, Kashlev M, Bustamante C. The elongation rate of RNA polymerase determines the fate of transcribed nucleosomes. *Nat Struct Mol Biol*. 2011; 18:1394–1399. [PubMed: 22081017]

- Bondarenko VA, Steele LM, Ujvari A, Gaykalova DA, Kulaeva OI, Polikanov YS, Luse DS, Studitsky VM. Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. *Mol Cell*. 2006; 24:469–479. [PubMed: 17081995]
- Brown CE, Lechner T, Howe L, Workman JL. The many HATs of transcription coactivators. *Trends Biochem Sci*. 2000; 25:15–19. [PubMed: 10637607]
- Brueckner F, Ortiz J, Cramer P. A movie of the RNA polymerase nucleotide addition cycle. *Curr Opin Struct Biol*. 2009; 19:294–299. [PubMed: 19481445]
- Burmann BM, Luo X, Rosch P, Wahl MC, Gottesman ME. Fine tuning of the E. coli NusB:NusE complex affinity to BoxA RNA is required for processive antitermination. *Nucleic Acids Res*. 2010; 38:314–326. [PubMed: 19854945]
- Burton ZF, Feig M, Gong XQ, Zhang C, Nedialkov YA, Xiong Y. NTP-driven translocation and regulation of downstream template opening by multi-subunit RNA polymerases. *Biochem Cell Biol*. 2005; 83:486–496. [PubMed: 16094452]
- Bushnell DA, Westover KD, Davis RE, Kornberg RD. Structural basis of transcription: an RNA polymerase II-TFIIBcocrystal at 4.5 Angstroms. *Science*. 2004; 303:983–988. [PubMed: 14963322]
- Chen CY, Chang CC, Yen CF, Chiu MT, Chang WH. Mapping RNA exit channel on transcribing RNA polymerase II by FRET analysis. *Proc Natl Acad Sci U S A*. 2009; 106:127–132. [PubMed: 19109435]
- Choi PJ, Cai L, Frieda K, Xie XS. A stochastic single-molecule event triggers phenotype switching of a bacterial cell. *Science*. 2008; 322:442–446. [PubMed: 18927393]
- Churchman LS, Weissman JS. Nascent transcript sequencing visualizes transcription at nucleotide resolution. *Nature*. 2011; 469:368–373. [PubMed: 21248844]
- Clapier CR, Cairns BR. The biology of chromatin remodeling complexes. *Annu Rev Biochem*. 2009; 78:273–304. [PubMed: 19355820]
- Close P, East P, Dirac-Svejstrup AB, Hartmann H, Heron M, Maslen S, Chariot A, Soding J, Skehel M, Svejstrup JQ. DBIRD complex integrates alternative mRNA splicing with RNA polymerase II transcript elongation. *Nature*. 2012; 484:386–389. [PubMed: 22446626]
- Comstock MJ, Ha T, Chemla YR. Ultrahigh-resolution optical trap with single-fluorophore sensitivity. *Nat Methods*. 2011; 8:335–340. [PubMed: 21336286]
- Conaway RC, Conaway JW. General transcription factors for RNA polymerase II. *Prog Nucleic Acid Res Mol Biol*. 1997; 56:327–346. [PubMed: 9187058]
- Cramer P, Bushnell DA, Kornberg RD. Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. *Science*. 2001; 292:1863–1876. [PubMed: 11313498]
- Dalal RV, Larson MH, Neuman KC, Gelles J, Landick R, Block SM. Pulling on the nascent RNA during transcription does not alter kinetics of elongation or ubiquitous pausing. *Mol Cell*. 2006; 23:231–239. [PubMed: 16857589]
- Darzacq X, Shav-Tal Y, de Turris V, Brody Y, Shenoy SM, Phair RD, Singer RH. In vivo dynamics of RNA polymerase II transcription. *Nat Struct Mol Biol*. 2007; 14:796–806. [PubMed: 17676063]
- de la Mata M, Alonso CR, Kadener S, Fededa JP, Blaustein M, Pelisch F, Cramer P, Bentley D, Kornblihtt AR. A slow RNA polymerase II affects alternative splicing in vivo. *Mol Cell*. 2003; 12:525–532. [PubMed: 14536091]
- Depken M, Galburt EA, Grill SW. The origin of short transcriptional pauses. *Biophys J*. 2009; 96:2189–2193. [PubMed: 19289045]
- Epshtein V, Nudler E. Cooperation between RNA polymerase molecules in transcription elongation. *Science*. 2003; 300:801–805. [PubMed: 12730602]
- Erie DA, Hajiseyedi O, Young MC, von Hippel PH. Multiple RNA polymerase conformations and GreA: control of the fidelity of transcription. *Science*. 1993; 262:867–873. [PubMed: 8235608]
- Foster JE, Holmes SF, Erie DA. Allosteric binding of nucleoside triphosphates to RNA polymerase regulates transcription elongation. *Cell*. 2001; 106:243–252. [PubMed: 11511351]
- Friedman LJ, Chung J, Gelles J. Viewing dynamic assembly of molecular complexes by multi-wavelength single-molecule fluorescence. *Biophys J*. 2006; 91:1023–1031. [PubMed: 16698779]

- Galburt EA, Grill SW, Wiedmann A, Lubkowska L, Choy J, Nogales E, Kashlev M, Bustamante C. Backtracking determines the force sensitivity of RNAP II in a factor-dependent manner. *Nature*. 2007; 446:820–823. [PubMed: 17361130]
- Gilchrist DA, Dos Santos G, Fargo DC, Xie B, Gao Y, Li L, Adelman K. Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation. *Cell*. 2010; 143:540–551. [PubMed: 21074046]
- Glover-Cutter K, Kim S, Espinosa J, Bentley DL. RNA polymerase II pauses and associates with pre-mRNA processing factors at both ends of genes. *Nat Struct Mol Biol*. 2008; 15:71–78. [PubMed: 18157150]
- Gnatt AL, Cramer P, Fu J, Bushnell DA, Kornberg RD. Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 Å resolution. *Science*. 2001; 292:1876–1882. [PubMed: 11313499]
- Gong XQ, Zhang C, Feig M, Burton ZF. Dynamic error correction and regulation of downstream bubble opening by human RNA polymerase II. *Mol Cell*. 2005; 18:461–470. [PubMed: 15893729]
- Greenleaf WJ, Frieda KL, Foster DA, Woodside MT, Block SM. Direct observation of hierarchical folding in single riboswitch aptamers. *Science*. 2008; 319:630–633. [PubMed: 18174398]
- Greenleaf WJ, Woodside MT, Block SM. High-Resolution, Single-Molecule Measurements of Biomolecular Motion. *Annu Rev Biophys Biomol Struct*. 2007; 36:171–190. [PubMed: 17328679]
- Guajardo R, Sousa R. A model for the mechanism of polymerase translocation. *J Mol Biol*. 1997; 265:8–19. [PubMed: 8995520]
- Gusarov I, Nudler E. The mechanism of intrinsic transcription termination. *Mol Cell*. 1999; 3:495–504. [PubMed: 10230402]
- Gutierrez-Medina B, Andreasson JO, Greenleaf WJ, Laporta A, Block SM. An optical apparatus for rotation and trapping. *Methods Enzymol*. 2010; 475:377–404. [PubMed: 20627165]
- Herbert KM, Greenleaf WJ, Block SM. Single-Molecule Studies of RNA Polymerase: Motoring Along. *Annu Rev of Biochem*. 2008; 77
- Herbert KM, La Porta A, Wong BJ, Mooney RA, Neuman KC, Landick R, Block SM. Sequence-resolved detection of pausing by single RNA polymerase molecules. *Cell*. 2006; 125:1083–1094. [PubMed: 16777599]
- Herbert KM, Zhou J, Mooney RA, Porta AL, Landick R, Block SM. E. coli NusG inhibits backtracking and accelerates pause-free transcription by promoting forward translocation of RNA polymerase. *J Mol Biol*. 2010; 399:17–30. [PubMed: 20381500]
- Hodges C, Bintu L, Lubkowska L, Kashlev M, Bustamante C. Nucleosomal fluctuations govern the transcription dynamics of RNA polymerase II. *Science*. 2009; 325:626–628. [PubMed: 19644123]
- Holmes SF, Erie DA. Downstream DNA sequence effects on transcription elongation. Allosteric binding of nucleoside triphosphates facilitates translocation via a ratchet motion. *J Biol Chem*. 2003; 278:35597–35608. [PubMed: 12813036]
- Hoskins AA, Friedman LJ, Gallagher SS, Crawford DJ, Anderson EG, Wombacher R, Ramirez N, Cornish VW, Gelles J, Moore MJ. Ordered and dynamic assembly of single spliceosomes. *Science*. 2011; 331:1289–1295. [PubMed: 21393538]
- Izban MG, Luse DS. The RNA polymerase II ternary complex cleaves the nascent transcript in a 3'→5' direction in the presence of elongation factor SII. *Genes Dev*. 1992; 6:1342–1356. [PubMed: 1378419]
- Jiang C, Pugh BF. Nucleosome positioning and gene regulation: advances through genomics. *Nat Rev Genet*. 2009; 10:161–172. [PubMed: 19204718]
- Jin J, Bai L, Johnson DS, Fulbright RM, Kireeva ML, Kashlev M, Wang MD. Synergistic action of RNA polymerases in overcoming the nucleosomal barrier. *Nat Struct Mol Biol*. 2010; 17:745–752. [PubMed: 20453861]
- Johnson RS, Strausbauch M, Cooper R, Register JK. Rapid kinetic analysis of transcription elongation by *Escherichia coli* RNA polymerase. *J Mol Biol*. 2008; 381:1106–1113. [PubMed: 18638485]
- Kaplan CD, Larsson KM, Kornberg RD. The RNA polymerase II trigger loop functions in substrate selection and is directly targeted by alpha-amanitin. *Mol Cell*. 2008; 30:547–556. [PubMed: 18538653]

- Kassavetis GA, Chamberlin MJ. Pausing and termination of transcription within the early region of bacteriophage T7 DNA in vitro. *J Biol Chem.* 1981; 256:2777–2786. [PubMed: 7009597]
- Kennedy SR, Erie DA. Templated nucleoside triphosphate binding to a noncatalytic site on RNA polymerase regulates transcription. *Proc Natl Acad Sci U S A.* 2011; 108:6079–6084. [PubMed: 21447716]
- Kettenberger H, Armache KJ, Cramer P. Complete RNA polymerase II elongation complex structure and its interactions with NTP and TFIIS. *Mol Cell.* 2004; 16:955–965. [PubMed: 15610738]
- Kireeva M, Kashlev M, Burton ZF. Translocation by multi-subunit RNA polymerases. *Biochim Biophys Acta.* 2010; 1799:389–401. [PubMed: 20097318]
- Kireeva ML, Hancock B, Cremona GH, Walter W, Studitsky VM, Kashlev M. Nature of the nucleosomal barrier to RNA polymerase II. *Mol Cell.* 2005; 18:97–108. [PubMed: 15808512]
- Kireeva ML, Kashlev M. Mechanism of sequence-specific pausing of bacterial RNA polymerase. *Proc Natl Acad Sci U S A.* 2009; 106:8900–8905. [PubMed: 19416863]
- Kireeva ML, Lubkowska L, Komissarova N, Kashlev M. Assays and affinity purification of biotinylated and nonbiotinylated forms of double-tagged core RNA polymerase II from *Saccharomyces cerevisiae*. *Methods Enzymol.* 2003; 370:138–155. [PubMed: 14712640]
- Kireeva ML, Nedialkov YA, Cremona GH, Purtov YA, Lubkowska L, Malagon F, Burton ZF, Strathern JN, Kashlev M. Transient reversal of RNA polymerase II active site closing controls fidelity of transcription elongation. *Mol Cell.* 2008; 30:557–566. [PubMed: 18538654]
- Komissarova N, Kashlev M. RNA polymerase switches between inactivated and activated states By translocating back and forth along the DNA and the RNA. *J Biol Chem.* 1997; 272:15329–15338. [PubMed: 9182561]
- Komissarova N, Kireeva ML, Becker J, Sidorenkov I, Kashlev M. Engineering of elongation complexes of bacterial and yeast RNA polymerases. *Methods Enzymol.* 2003; 371:233–251. [PubMed: 14712704]
- Kulaeva OI, Hsieh FK, Studitsky VM. RNA polymerase complexes cooperate to relieve the nucleosomal barrier and evict histones. *Proc Natl Acad Sci U S A.* 2010; 107:11325–11330. [PubMed: 20534568]
- La Porta A, Wang MD. Optical torque wrench: angular trapping, rotation, and torque detection of quartz microparticles. *Phys Rev Lett.* 2004; 92:190801. [PubMed: 15169392]
- Landick R, Carey J, Yanofsky C. Translation activates the paused transcription complex and restores transcription of the trp operon leader region. *Proc Natl Acad Sci U S A.* 1985; 82:4663–4667. [PubMed: 2991886]
- Lang MJ, Fordyce PM, Engh AM, Neuman KC, Block SM. Simultaneous, coincident optical trapping and single-molecule fluorescence. *Nat Methods.* 2004; 1:133–139. [PubMed: 15782176]
- Larson DR, Zenklusen D, Wu B, Chao JA, Singer RH. Real-time observation of transcription initiation and elongation on an endogenous yeast gene. *Science.* 2011; 332:475–478. [PubMed: 21512033]
- Larson MH, Zhou J, Kaplan CD, Palangat M, Kornberg RD, Landick R, Block SM. Trigger loop dynamics mediate the balance between the transcriptional fidelity and speed of RNA polymerase II. *Proc Natl Acad Sci U S A.* 2012
- Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD. Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat Genet.* 2004; 36:900–905. [PubMed: 15247917]
- Lin C, Garrett AS, De Kumar B, Smith ER, Gogol M, Seidel C, Krumlauf R, Shilatifard A. Dynamic transcriptional events in embryonic stem cells mediated by the super elongation complex (SEC). *Genes Dev.* 2011; 25:1486–1498. [PubMed: 21764852]
- Martinez-Rucobo FW, Sainsbury S, Cheung AC, Cramer P. Architecture of the RNA polymerase-Spt4/5 complex and basis of universal transcription processivity. *EMBO J.* 2011; 30:1302–1310. [PubMed: 21386817]
- Min IM, Waterfall JJ, Core LJ, Munroe RJ, Schimenti J, Lis JT. Regulating RNA polymerase pausing and transcription elongation in embryonic stem cells. *Genes Dev.* 2011; 25:742–754. [PubMed: 21460038]
- Muschielok A, Andrecka J, Jawhari A, Bruckner F, Cramer P, Michaelis J. A nano-positioning system for macromolecular structural analysis. *Nat Methods.* 2008; 5:965–971. [PubMed: 18849988]

- Muse GW, Gilchrist DA, Nechaev S, Shah R, Parker JS, Grissom SF, Zeitlinger J, Adelman K. RNA polymerase is poised for activation across the genome. *Nat Genet.* 2007; 39:1507–1511. [PubMed: 17994021]
- Nechaev S, Fargo DC, dos Santos G, Liu L, Gao Y, Adelman K. Global analysis of short RNAs reveals widespread promoter-proximal stalling and arrest of Pol II in *Drosophila*. *Science.* 2010; 327:335–338. [PubMed: 20007866]
- Nedialkov YA, Gong XQ, Hovde SL, Yamaguchi Y, Handa H, Geiger JH, Yan H, Burton ZF. NTP-driven translocation by human RNA polymerase II. *J Biol Chem.* 2003; 278:18303–18312. [PubMed: 12637520]
- Neuman KC, Abbondanzieri EA, Landick R, Gelles J, Block SM. Ubiquitous transcriptional pausing is independent of RNA polymerase backtracking. *Cell.* 2003; 115:437–447. [PubMed: 14622598]
- Palangat M, Larson MH, Hu X, Gnatt A, Block SM, Landick R. Efficient reconstitution of transcription elongation complexes for single-molecule studies of eukaryotic RNA polymerase II. *Transcription.* 2012; 3:1–8.
- Palangat M, Meier TI, Keene RG, Landick R. Transcriptional pausing at +62 of the HIV-1 nascent RNA modulates formation of the TAR RNA structure. *Mol Cell.* 1998; 1:1033–1042. [PubMed: 9651586]
- Pan T, Sosnick T. RNA folding during transcription. *Annu Rev Biophys Biomol Struct.* 2006; 35:161–175. [PubMed: 16689632]
- Peterlin BM, Price DH. Controlling the elongation phase of transcription with P-TEFb. *Mol Cell.* 2006; 23:297–305. [PubMed: 16885020]
- Plant KE, Dye MJ, Lafaille C, Proudfoot NJ. Strong polyadenylation and weak pausing combine to cause efficient termination of transcription in the human γ -globin gene. *Mol Cell Biol.* 2005; 25:3276–3285. [PubMed: 15798211]
- Proshkin S, Rahmouni AR, Mironov A, Nudler E. Cooperation between translating ribosomes and RNA polymerase in transcription elongation. *Science.* 2010; 328:504–508. [PubMed: 20413502]
- Rahl PB, Lin CY, Seila AC, Flynn RA, McCuine S, Burge CB, Sharp PA, Young RA. c-Myc regulates transcriptional pause release. *Cell.* 2010; 141:432–445. [PubMed: 20434984]
- Rhee HS, Pugh BF. Comprehensive genome-wide protein-DNA interactions detected at single-nucleotide resolution. *Cell.* 2011; 147:1408–1419. [PubMed: 22153082]
- Rhee HS, Pugh BF. Genome-wide structure and organization of eukaryotic pre-initiation complexes. *Nature.* 2012; 483:295–301. [PubMed: 22258509]
- Richardson JP. Rho-dependent termination and ATPases in transcript termination. *Biochim Biophys Acta.* 2002; 1577:251–260. [PubMed: 12213656]
- Schafer DA, Gelles J, Sheetz MP, Landick R. Transcription by single molecules of RNA polymerase observed by light microscopy. *Nature.* 1991; 352:444–448. [PubMed: 1861724]
- Schwabish MA, Struhl K. The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation in vivo. *Mol Cell Biol.* 2007; 27:6987–6995. [PubMed: 17709398]
- Shaevitz JW, Abbondanzieri EA, Landick R, Block SM. Backtracking by single RNA polymerase molecules observed at near-base-pair resolution. *Nature.* 2003; 426:684–687. [PubMed: 14634670]
- Shukla S, Kavak E, Gregory M, Imashimizu M, Shutinoski B, Kashlev M, Oberdoerffer P, Sandberg R, Oberdoerffer S. CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. *Nature.* 2011; 479:74–79. [PubMed: 21964334]
- Studitsky VM, Clark DJ, Felsenfeld G. A histone octamer can step around a transcribing polymerase without leaving the template. *Cell.* 1994; 76:371–382. [PubMed: 8293470]
- Studitsky VM, Kassavetis GA, Geiduschek EP, Felsenfeld G. Mechanism of transcription through the nucleosome by eukaryotic RNA polymerase. *Science.* 1997; 278:1960–1963. [PubMed: 9395401]
- Thiriet C, Hayes JJ. Replication-independent core histone dynamics at transcriptionally active loci in vivo. *Genes Dev.* 2005; 19:677–682. [PubMed: 15769942]
- Touloukhonov I, Zhang J, Palangat M, Landick R. A central role of the RNA polymerase trigger loop in active-site rearrangement during transcriptional pausing. *Mol Cell.* 2007; 27:406–419. [PubMed: 17679091]

- Ujvari A, Luse DS. RNA emerging from the active site of RNA polymerase II interacts with the Rpb7 subunit. *Nat Struct Mol Biol.* 2006; 13:49–54. [PubMed: 16327806]
- Ujvari A, Pal M, Luse DS. The functions of TFIIF during initiation and transcript elongation are differentially affected by phosphorylation by casein kinase 2. *J Biol Chem.* 2011; 286:23160–23167. [PubMed: 21566144]
- Vassilyev DG, Vassilyeva MN, Perederina A, Tahirov TH, Artsimovitch I. Structural basis for transcription elongation by bacterial RNA polymerase. *Nature.* 2007a; 448:157–162. [PubMed: 17581590]
- Vassilyev DG, Vassilyeva MN, Zhang J, Palangat M, Artsimovitch I, Landick R. Structural basis for substrate loading in bacterial RNA polymerase. *Nature.* 2007b; 448:163–168. [PubMed: 17581591]
- Walter W, Kireeva ML, Studitsky VM, Kashlev M. Bacterial polymerase and yeast polymerase II use similar mechanisms for transcription through nucleosomes. *J Biol Chem.* 2003; 278:36148–36156. [PubMed: 12851391]
- Wang D, Bushnell DA, Huang X, Westover KD, Levitt M, Kornberg RD. Structural basis of transcription: backtracked RNA polymerase II at 3.4 angstrom resolution. *Science.* 2009; 324:1203–1206. [PubMed: 19478184]
- Wang D, Bushnell DA, Westover KD, Kaplan CD, Kornberg RD. Structural basis of transcription: role of the trigger loop in substrate specificity and catalysis. *Cell.* 2006; 127:941–954. [PubMed: 17129781]
- Wang MD, Schnitzer MJ, Yin H, Landick R, Gelles J, Block SM. Force and velocity measured for single molecules of RNA polymerase. *Science.* 1998; 282:902–907. [PubMed: 9794753]
- Westover KD, Bushnell DA, Kornberg RD. Structural basis of transcription: nucleotide selection by rotation in the RNA polymerase II active center. *Cell.* 2004a; 119:481–489. [PubMed: 15537538]
- Westover KD, Bushnell DA, Kornberg RD. Structural basis of transcription: separation of RNA from DNA by RNA polymerase II. *Science.* 2004b; 303:1014–1016. [PubMed: 14963331]
- Wickiser JK, Winkler WC, Breaker RR, Crothers DM. The speed of RNA transcription and metabolite binding kinetics operate an FMN riboswitch. *Mol Cell.* 2005; 18:49–60. [PubMed: 15808508]
- Yin H, Wang MD, Svoboda K, Landick R, Block SM, Gelles J. Transcription against an applied force. *Science.* 1995; 270:1653–1657. [PubMed: 7502073]
- Yin YW, Steitz TA. The structural mechanism of translocation and helicase activity in T7 RNA polymerase. *Cell.* 2004; 116:393–404. [PubMed: 15016374]
- Yonaha M, Proudfoot NJ. Specific transcriptional pausing activates polyadenylation in a coupled in vitro system. *Mol Cell.* 1999; 3:593–600. [PubMed: 10360175]
- Yuzenkova Y, Bochkareva A, Tadigotla VR, Roghanian M, Zorov S, Severinov K, Zenkin N. Stepwise mechanism for transcription fidelity. *BMC Biol.* 2010; 8:54. [PubMed: 20459653]
- Zawel L, Kumar KP, Reinberg D. Recycling of the general transcription factors during RNA polymerase II transcription. *Genes Dev.* 1995; 9:1479–1490. [PubMed: 7601352]
- Zhang C, Burton ZF. Transcription factors IIF and IIS and nucleoside triphosphate substrates as dynamic probes of the human RNA polymerase II mechanism. *J Mol Biol.* 2004; 342:1085–1099. [PubMed: 15351637]
- Zhou J, Ha KS, La Porta A, Landick R, Block SM. Applied force provides insight into transcriptional pausing and its modulation by transcription factor NusA. *Mol Cell.* 2011; 44:635–646. [PubMed: 22099310]
- Zhou Q, Li T, Price DH. RNA Polymerase II Elongation Control. *Annu Rev Biochem.* 2012

Figure 1.

Figure 2.

Figure 3.

Figure 4.