Transcription factors IIS and IIF enhance transcription efficiency by differentially modifying RNA polymerase pausing dynamics

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Transcription factors IIS (TFIIS) and IIF (TFIIF) are known to stimulate transcription elongation. Here, we use a single-molecule transcription elongation assay to study the effects of both factors. We find that these transcription factors enhance overall transcription elongation by reducing the lifetime of transcriptional pauses and that TFIIF also decreases the probability of pause entry. Furthermore, we observe that both factors enhance the processivity of RNA polymerase II through the nucleosomal barrier. The effects of TFIIS and TFIIF are quantitatively described using the linear Brownian ratchet kinetic model for transcription elongation and the backtracking model for transcriptional pauses, modified to account for the effects of the transcription factors. Our findings help elucidate the molecular mechanisms by which transcription factors modulate gene expression.

optical tweezers | Pol II | yeast | enzyme kinetics

Transcription regulation is the first step in the control of gene expression and it is a fundamental and highly coordinated process in all cells. RNA polymerase II (Pol II) is responsible for the synthesis of mRNAs, most snRNAs, and microRNAs in eukaryotic cells. Transcription elongation by Pol II is regulated by many elements such as the state of Pol II phosphorylation in the C-terminal repeat domain (CTD), the presence and stability of nucleosomes, the extent and stability of the nascent RNA structure formed behind Pol II, and several transcription factors. However, many of the molecular details underlying Pol II transcriptional regulation remain unknown.

When Pol II synthesizes an RNA transcript, the enzyme translocates along the DNA template by thermally fluctuating between the pre- and the posttranslocated states; the binding of NTP to the posttranslocated state rectifies the forward motion in a mechanism that is consistent with Pol II operating as a Brownian ratchet (1–3). After the binding of an NTP, Pol II rapidly hydrolyzes the NTP, extends the nascent RNA transcript by 1 nt, and releases pyrophosphate (PPi). During transcription elongation, Pol II is also susceptible to entering a paused state, which is a major regulatory element for transcriptional repression (4). In a paused state, Pol II is known to backtrack wherein the 3' end of the nascent RNA transcript is extruded from its active site. Backtracked Pol II molecules remain catalytically inactive and may become transcriptionally competent only when the enzyme restores the registry between the Pol II's active site and the 3' end of the transcript either by diffusion forward along the DNA template or by cleaving of the misaligned transcript at the backtracked position of the active site (1, 5).

Many transcription factors, including TFIIS and TFIIF, are known to regulate transcription elongation by directly interacting with the polymerase (6). TFIIS rescues backtracked Pol II molecules by stimulating the intrinsic endonucleolytic activity of Pol II (7). An internal scission of the RNA backbone removes 2-nt or longer fragments of the nascent RNA and returns the enzyme to a posttranslocated state, from which it resumes transcription

elongation (8). Misincorporated nucleotides favor backtracking of the enzyme; thus, TFIIS-induced cleavage promotes transcription fidelity both in vitro and in vivo (9–11).

TFIIF, by contrast, has an established role in transcription initiation, where it associates with Pol II and five other general transcription factors to form the preinitiation complex. TFIIF is necessary for the recruitment of Pol II to the preinitiation complex, and it either recruits or retains TFIIB during transcription initiation (12–15). A recent cryo-EM reconstruction of the preinitiation complex of human Pol II suggests that TFIIF can stabilize the downstream DNA along the cleft of the enzyme (16), in addition to stabilizing the RNA–DNA hybrid within the polymerase (14). TFIIF is also involved in the elongation phase of transcription in vivo in both yeast (17) and mammals (15). TFIIF is known to stimulate the overall elongation rate in mammalian systems (18–20); however, the detailed mechanism by which TFIIF affects the elongation phase is still unknown. Moreover, although TFIIF is known to bind elongating Pol II in yeast (17), its effects on the elongation process have not been demonstrated.

Transcription elongation in eukaryotic cells is also regulated by the presence of histones that organize the DNA in the form of

Significance

Regulation of gene expression controls fundamental cellular processes, such as growth and differentiation. Gene expression begins at transcription, in which the eukaryotic RNA polymerase (Pol II) synthesizes the precursors of mRNA during the elongation phase. The speed and fidelity of the process are regulated by many transcription elongation factors, including transcription factors IIS (TFIIS) and IIF (TFIIF), which directly interact with the enzyme. Using a single-molecule optical-tweezers assay, we have quantified the effects of these factors on the dynamics of transcription elongation by Pol II on both bare and nucleosomal DNA. We showed that TFIIF mainly prevents Pol II from pause entering, whereas TFIIS assists with pause recovery, and quantitatively described these effects on the kinetics of transcription elongation by Pol II.

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nucleosomes. The nucleosome presents a physical barrier that prevents the progression of Pol II along the DNA template (21–24). Gel-based biochemical assays have shown that TFIIS strongly stimulates *in vitro* transcription through a single nucleosome (21) and even chromatin templates containing multiple nucleosomes (25, 26). In a mammalian transcription system, TFIIF has been shown to enhance nucleosomal passage of Pol II, and the presence of both TFIIS and TFIIF significantly improves the efficiency of passage (27). It remains unclear whether similar effects would occur in the yeast enzyme.

We investigated the effects of yeast TFIIS and TFIIF on transcription elongation by Pol II, using a single-molecule optical-tweezers assay, which follows transcription dynamics in real time and allows for separation of off-pathway transcriptional pauses from on-pathway elongation. We found that the presence of either factor did not substantially affect the pause-free velocity of the enzyme (i.e., its active elongation rate without pauses); instead, both factors regulate transcriptional pauses. In particular, TFIIS shortens the time Pol II spends in the paused state, whereas TFIIF enhances transcription elongation by decreasing the probability of pausing and shortening the pause durations in a force-dependent manner. The effects of these factors on transcription of bare DNA were also observed on transcription of nucleosomal DNA. In the latter case, they were seen to affect the elongation dynamics as well as the nucleosomal passage probability. Our results indicate that the main mechanism by which these factors regulate Pol II transcription elongation is through changes in the enzyme pausing dynamics.

Results

Single-Molecule Optical-Tweezers Transcription Elongation Assay in the Presence of Transcription Factors. To investigate the effects of transcription factors during Pol II elongation, we used optical tweezers to apply force and monitor the position of Pol II along the DNA template in real time. A DNA tether was created in an opposing force configuration by attaching a stalled biotinylated Pol II elongation complex to a streptavidin (SA) bead and the digoxigenin-labeled downstream end of the DNA template to an anti-digoxigenin (AD) bead (Fig. 1A) (28). Alternatively, we switched the direction of the applied force to assist transcription elongation by labeling the upstream end of the DNA with a digoxigenin molecule and attaching it to an AD bead (assisting force configuration). Transcription factors (TFIIS, TFIIF, or both) were introduced to the sample chamber at the same time with NTP substrates. The overall elongation of Pol II in the presence of TFIIF (7.7 ± 0.8 nt/s) or TFIIS (7.3 ± 1.3 nt/s) is faster than that in the absence of the factors (4.9 ± 0.8 nt/s) (Fig. 1B and Table S1). These rates were measured in passive mode under 4–7 pN of opposing loads; errors are SEM unless otherwise specified. Transcription elongation is punctuated by pauses, which can be separated from active elongation to obtain pause-free velocities. The mean pause-free velocities are 19 ± 2 nt/s for Pol II in the absence of transcription factors (Fig. 1D), in good agreement with previously published results (29, 30); 21 ± 2 nt/s with TFIIS; and 23 ± 2 nt/s with TFIIF (opposing force range of 4–7 pN).

Our observation that the pause-free velocities of actively transcribing Pol II cannot account for the overall increase in elongation velocity suggests that another part of the kinetic pathway, namely the off-pathway pausing, plays a significant role in explaining the effects of these transcription factors. Indeed, transcriptional pauses become significantly shorter in the presence of TFIIS in both opposing and assisting force configurations [Kolmogorov–Smirnov (K-S) test: opposing force in Fig. 1F, $P = 0.096$; and assisting force in Fig. S1, $P \sim 0.01$]. The number of pauses detected (pauses lasting between 1 s and 120 s) also decreases, presumably because more pauses become shorter than our detection limit of 1 s (Fig. 1E). Moreover, TFIIS also increased the stall force of Pol II from 6.7 ± 0.4 pN to 9.0 ± 0.8 pN (Fig. S2). These forces are somewhat different from those reported previously (28). It is known, however, that the stall

Fig. 1. Single-molecule transcription elongation in the presence of transcription factors TFIIS and TFIIF. (A) Experimental geometry for the single-molecule transcription assay in opposing force configuration. (B) Example traces of Pol II transcription in the absence of factors (black), with TFIIS (blue), with TFIIF (red), and with TFIIF/TFIIS (green). (C) An enlarged typical example trace of Pol II transcription with TFIIS (Left) and TFIIF (Right) at high opposing force (~ 10 pN). (D) Pause-free velocity against applied forces. Error bars represent SEMs. Note that we excluded the apparent pause density of Pol II in the 7-pN to 10-pN range in the plot because the mean stall force of Pol II is about 7 pN. (E) Mean pause densities of Pol II in the absence and presence of transcription factors within the force range studied. Vertical lines represent SEMs. (F) The cumulative pause duration distributions of Pol II (black line) and Pol II with TFIIS (blue line) in the 4-pN to 7-pN opposing force range. Dashed lines represent fits from the models (Fig. 3). (G) The cumulative pause duration distributions of Pol II (black line), Pol II with TFIIF (red line), and Pol II with TFIIF/TFIIS (green line) in the 4-pN to 7-pN opposing force range.

forces will change with the DNA templates because of the differences in the stability of the nascent RNA secondary structures, determined in part by the GC content (31). The template used in this study has a uniformly lower GC content than that used by Galbur et al. (Fig. S3) (28); thus, we attribute differences in observed stall forces in part to RNA structure. In addition, the stall force in the presence of TFIIS reported here likely represents a lower bound estimate of the effect of this factor. Despite a saturating concentration of TFIIS [$2 \mu\text{M}$, 20 times greater than the K_d (32)], it is possible that TFIIS does not bind to all Pol II molecules throughout the elongation process, as noted by Galbur et al. (28). At forces much higher than the mean stall force, Pol II typically backtracks over a large distance and cannot resume transcription from these backtracks. However, in the presence of TFIIS, we observed that the factor can rescue Pol II molecules that have backtracked more than 10 bp (Fig. 1C). These real-time observations agree with previous experiments suggesting that TFIIS enhances Pol II recovery from backtracked pauses by stimulating its endonucleolytic activity (7,

28). The observed increase in stall force can also be explained in terms of TFIIS-stimulated rescue: As a fraction of backtracked Pol II molecules are rescued in the presence of TFIIS, Pol II is more likely to transcribe against higher opposing loads (28).

As with TFIIS, TFIIF similarly decreased the number of pauses detected in both opposing and assisting force geometries (Fig. 1E). TFIIF shortened the duration of pauses in the assisting force configuration ($P = 0.014$, Fig. S1). However, this effect in the distribution of pause durations was not detected in the opposing force configuration ($P \sim 0.5$, Fig. 1G). Taken together, these results reveal that TFIIF also regulates transcription elongation by modifying the pausing dynamics of Pol II. As would be expected for a transcription factor that decreases the probability that Pol II enters a paused state (thus making Pol II less susceptible to transcriptional arrest), the stall force of Pol II also increases in the presence of TFIIF: 8.0 ± 0.4 pN, compared with 6.7 ± 0.4 pN for Pol II alone (Fig. S2).

Having established the effects on transcription elongation of each individual factor, we sought to characterize Pol II dynamics in the presence of both TFIIS and TFIIF, as may occur in vivo. We found that the distribution of pause durations in the presence of both transcription factors does not differ significantly from that observed in the presence of either TFIIF or TFIIS alone [(K-S test, $\alpha = 0.05$) Fig. 1G and Fig. S1, opposing and assisting force configurations, respectively]. Furthermore, the apparent pause density in the presence of both transcription factors is lower than that observed in the presence of either transcription factor alone only in the lower opposing force range studied (Fig. 1E). Finally, the stall force in the presence of both TFIIS and TFIIF was 9.8 ± 0.5 pN compared with 9.0 ± 0.8 pN and 8.0 ± 0.4 pN in the presence of only TFIIS and only TFIIF, respectively. Our results indicate that there is a weak enhancement of transcription elongation when both transcription factors are present simultaneously relative to the enhancement observed when either factor is present alone.

Pol II Transcription Through a Nucleosome. In eukaryotic cells, genomic DNA is wrapped into nucleosomes, which regulate transcription by acting as a barrier to Pol II elongation (21, 22, 33, 34). Therefore, defining the mechanisms by which transcription factors assist Pol II elongation through nucleosomes lies at the heart of understanding transcription regulation in the cell.

To this end, we used assisting force geometry with the downstream DNA template harboring a nucleosome whose position was defined by a 601 nucleosomal positioning sequence (NPS). We verified that the transcription templates were saturated with single nucleosomes at the correct position, as assayed by a native gel-electrophoresis assay (Fig. S4) (22). In the presence of either transcription factor, we observed that Pol II spends less time at the entry of the nucleosome (-115 bp to -35 bp with respect to the nucleosome dyad) and that Pol II transcribes nucleosomal DNA more efficiently, as reflected by the probabilities of nucleosomal passage (Fig. 2 and Table S1). In 300 mM KCl, 63% of Pol II molecules (59 of 93 molecules in total) were found to pass through the nucleosome in the absence of any transcription factor, 74% in the presence of TFIIS (42 of 57 molecules), 72% in the presence of TFIIF (23 of 32 molecules), and 77% when both TFIIS and TFIIF (18 of 23 molecules) were present (Fig. 2C). Our results indicate that one factor may interfere with the other's function; hence, we did not observe a quantitative addition of their effects.

Mechanistically, the nucleosome acts as a rapidly fluctuating barrier that allows the polymerase to progress only when it is unwrapped in front of the enzyme. In the absence of TFIIS, a backtracked Pol II must wait for DNA unwrapping before it can diffuse forward and recover from a backtracked pause (22). In the presence of TFIIS, however, RNA cleavage places Pol II in the elongation-competent state, rescuing the enzyme from a backtracked pause and thus facilitating transcription through a nucleosome. The effect of TFIIF, namely preventing pause entering and

Fig. 2. Transcription factors TFIIF and TFIIS enhance Pol II elongation through the nucleosome. (A) Example traces of Pol II transcription on nucleosomal DNA in the absence of factors (black), with TFIIF (red), with TFIIS (blue), and with TFIIF/TFIIS (green). Some molecules stop in the nucleosome region (right side). (B) Mean dwell times against distance from the nucleosomal dyad. Error bars represent SEMs. (C) Histograms of transcription arrest sites in the presence of different factors. Numbers are the percentage of Pol II molecules that passed through the nucleosome. The extended NPS region (-115 nt to $+85$ nt) is highlighted in yellow. All nucleosomal transcription experiments were done under an assisting load (Methods).

reducing pause durations of Pol II, can similarly explain how this factor facilitates transcription through a nucleosome (35, 36).

We observed that the presence of transcription factors does not substantially affect the pause-free velocities of Pol II even in the nucleosome region (Fig. S5). This result is consistent with the pausing dynamics being responsible for Pol II's enhanced processivity through the nucleosome. Although TFIIS and TFIIF function via different mechanisms (Discussion), both factors favor the on-pathway phase, reducing the probability of arrest and, consequently, increasing nucleosomal passage.

A Kinetic Model That Explains the Effects of Transcription Factors. To quantitatively describe the observed effects established here for the transcription factors, we modified the recently reported linear Brownian ratchet model for Pol II elongation (30) (Fig. 3) to account for the effects of transcription factors. Briefly, at each nucleotide position along the DNA template, the pretranslocated Pol II can either transit to the posttranslocated state and incorporate a nucleotide (the "on-pathway" mechanism, green in Fig. 3) or enter a pause ("off-pathway" mechanism, purple in Fig. 3). In the on-pathway mechanism, Pol II thermally fluctuates between the pretranslocated state (denoted $TEC_{n,0}$) and the posttranslocated state ($TEC_{n,1}$). By convention, the first sub-index (n) corresponds to the RNA transcript length and the second sub-index indicates the translocation state (0 for "pre" or 1 for "post"). Translocation by Pol II occurs with a forward rate, k_1 , and a backward rate, k_{-1} . Once in the posttranslocated state, NTP can bind to the active site and rectify the forward translocation with the NTP binding (k_2) and dissociation rates (k_{-2}). After NTP binding, the enzyme catalyzes the phosphodiester bond formation with nascent RNA and releases PPi; we represent the combined catalysis rate constant that includes bond formation and PPi release by the rate k_3 . Pol II then completes a cycle of nucleotide addition, moves forward on the DNA by 1 bp, and returns to the pretranslocated state with one additional nucleotide in the RNA transcript ($TEC_{n+1,0}$). At each position along the template, Pol II may also enter into a pause with the rate k_{b1} , thus kinetically competing with a forward translocation (with rate k_1). If Pol II backtracks, it enters the off-pathway pausing states (purple in Fig. 3). There, Pol II diffuses along the DNA template with force-biased diffusion rate constants in the

Fig. 3. Kinetic model of transcription elongation by Pol II in the presence of TFIIS. Transcription elongation by Pol II is composed of the on-pathway elongation (green) and the off-pathway pausing (purple). Forward translocation (k_1) competes with entry into backtracked pauses (k_{b1}). In the absence of TFIIS, pause recovery requires forward diffusion of the enzyme (k_f) to a pretranslocated state such as $TEC_{n,0}$. TFIIS introduces a new pause recovery mechanism (k_r , red arrows) that takes a backtracked Pol II in the state $TEC_{i,-j}$ to the on-pathway posttranslocated state $TEC_{i-j+1,1}$. Cartoon configurations of Pol II TECs in the pre- and posttranslocated and 1-bp backtracked states show that TFIIS-stimulated transcript cleavage rescues the 1-bp backtracked Pol II complex ($TEC_{n,-1}$), transferring it to the elongation-competent posttranslocated state $TEC_{n-2,1}$. The purple arrow represents the active site of the enzyme. The RNA transcript and template DNA are shown in red and blue, respectively. N represents NTP.

forward (k_f) and backward (k_b) movements (downstream and upstream in Fig. 3, respectively), given by

$$k_f = k_0 e^{F \cdot d / k_B T} \quad [1]$$

$$k_b = k_0 e^{-(F \cdot d + \delta G_{RNA}) / k_B T} \quad [2]$$

Here, k_0 is the intrinsic rate constant describing Pol II diffusion along DNA during backtracking at zero force, d is the distance to the transition state for each step (taken here to be 0.5 bp), F is the applied external force, and δG_{RNA} is an energy barrier to backtracking due to the nascent RNA secondary structure behind Pol II (31). In a paused state, Pol II performs a random walk back and forth along the DNA until the 3' end of the RNA restores registration with the active site of Pol II (28). Therefore, the pause durations can be modeled as the first passage times of a 1D random walker and the probability density of pause durations, $\psi(t)$, is then given by

$$\psi(t) = \sqrt{\frac{k_f}{k_b}} \frac{\exp[-(k_f + k_b)t]}{t} I_1(2t\sqrt{k_f k_b}), \quad [3]$$

where I_1 is the modified Bessel function of the first kind (22, 37). The pause density ρ_{pause} (in bp^{-1}) reflects the probability of entering a pause, which results from the competition between the first backtracking step (k_{b1}) and the net forward translocation. At saturating NTP concentrations, such as in our experimental condition (1 mM), k_1 can adequately describe the net rate of forward translocation (Fig. 3) (30); thus $\rho_{\text{pause(sat)}}$ can be written as

$$\rho_{\text{pause(sat)}} = \frac{k_{b1}}{k_{b1} + k_1} \quad [4]$$

We have counted only pauses with lifetimes between 1 s and 120 s in our experiments. To relate the theoretical absolute pause

density given by Eq. 4 to the experimental result, the theoretical density must be multiplied by the fraction of pauses that are within the pause detection limits (1–120 s). The theoretical apparent pause density in the range of 1–120 s can then be calculated by

$$\rho_{\text{pause(sat)}, 1 < t < 120} = \rho_{\text{pause(sat)}} \cdot \int_1^{120} \psi(t) dt. \quad [5]$$

We fitted our opposing pause density and pause duration data in the absence of transcription factors based on this model. Using previously reported rates for transcription by Pol II at zero force, $k_{b1} = 8.0 \text{ s}^{-1}$ and $k_1 = 88 \text{ s}^{-1}$ (30), we extracted the rate $k_0 = 1.6 \pm 0.3 \text{ s}^{-1}$ and $\delta G_{RNA} = 1.1 \pm 0.2 k_B T$, using the expressions for the pause duration distribution and apparent pause density (Eqs. 3 and 5, respectively) (31).

In the presence of TFIIS, we observed that pause durations became shorter due to the TFIIS-stimulated endonucleolytic activity of Pol II (8, 28). To quantitatively address this effect, we introduced an additional backtracked pause recovery pathway to the kinetic scheme described above (Fig. 3, red arrows). This new recovery pathway takes Pol II from any off-pathway, backtracked state ($TEC_{i,-j}$, where the second negative subindex indicates a backtracked state and j indicates the number of base pairs backtracked) to the on-pathway posttranslocated state ($TEC_{i-j+1,1}$) with a recovery rate k_r (Fig. 3) as a result of the $(j+1)$ -nt RNA transcript cleavage. For instance, in Fig. 3, this pause recovery pathway takes a 1-bp backtracked Pol II molecule ($TEC_{n+1,-1}$) to the posttranslocated state ($TEC_{n-1,1}$) after a 2-nt RNA transcript cleavage (red arrow). The corresponding configurations of Pol II transcription elongation complexes are highlighted in Fig. 3. In the presence of TFIIS, two possible mechanisms exist to rescue the enzyme from a backtracked pause state: the return of Pol II to the transcription-competent on-pathway state by diffusion or the TFIIS-stimulated endonucleolytic cleavage. While Pol II is in the backtracked state, the forward and backward diffusive movements compete with TFIIS-stimulated backtrack recovery.

Note that the introduction of the rate k_r effectively shortens pause durations compared with those in the original model (i.e., where $k_r = 0 \text{ s}^{-1}$). Accordingly, a pause can no longer be described as a random walk of the enzyme along DNA with return to the pretranslocated state; consequently, the probability density of pause durations can no longer be described by Eq. 3. To determine the rate k_r , we performed a kinetic Monte Carlo simulation of the kinetic mechanism shown in Fig. 3 (*SI Methods*). Fitting the experimental pause durations and densities to our model, we extracted a recovery rate $k_r = 0.4 \pm 0.2 \text{ s}^{-1}$. Fig. 1*F* compares the experimental pause duration distribution in the presence of TFIIS with the theoretical prediction obtained with this k_r value. This simulation shows that the decreased pause density observed in the presence of TFIIS can be rationalized by the increased fraction of pauses that become shorter than our experimental detection limit (1 s). The extracted value of the k_r rate is on the same order of magnitude as the endonucleolytic rate $k_{clv} = 0.1 \text{ s}^{-1}$ recently estimated (38).

In the case of TFIIF, we observed both reductions in the pause density for Pol II under opposing and assisting forces and reductions in pause duration only under assisting force conditions. The reduced pause density can in principle be explained by an increased forward translocation rate (k_1), a decreased rate of pause entry (k_{b1}), or both (Eq. 4). From the pause density observed in the presence of TFIIF, we estimate that if this factor affected only k_{b1} , then this rate would have to decrease from 8.0 s^{-1} to 5.7 s^{-1} . If, on the other hand, TFIIF increased only k_1 , then k_1 would have to rise from 88 s^{-1} to 124 s^{-1} . Note that these values are force dependent, and reported here are average rates extrapolated to zero force. Also note that the changes in k_1 required to explain the reduced pause density would not lead to an observable change in pause-free velocity, given the variance of our data. Moreover, changes to either or both of these rates would not explain the decrease in pause duration observed under assisting loads. For this observation, our model suggests various possible scenarios. Mechanistically, shorter pause durations could arise from a higher rate of forward diffusion (k_f), a reduced rate of backward diffusion (k_b), a combination of the two, or a higher rate of intrinsic diffusion (k_0). At present, we cannot distinguish among these possibilities (*Discussion*).

Discussion

Biochemical characterizations of TFIIS have shown that it enhances the intrinsic endonucleolytic activity of Pol II. Here, we observed that TFIIS stimulates transcription elongation by shortening the durations of transcriptional pauses, without affecting the pause-free velocity. From these observations, we have measured the TFIIS-stimulated rate of recovery from a paused state (k_r) to be $0.4 \pm 0.2 \text{ s}^{-1}$. The TFIIS-stimulated recovery positions the enzyme in the posttranslocated state, from which Pol II can resume active elongation, eliminating the required diffusional search of the enzyme for the 3' end. Hence, by modulating the pausing dynamics, TFIIS strongly increases the efficiency of transcription elongation. Note that our assay does not measure RNA transcript cleavage directly, but instead monitors transcription elongation resumption. Therefore, the TFIIS-induced recovery described by the rate k_r does not necessarily equal the rate of cleavage of the nascent RNA at Pol II's active site. The pause recovery process may also include other rates such as diffusion of the cleaved RNA transcript out of the enzyme and conformational changes of Pol II required to resume active transcription elongation. Note also that the kinetic model presented here considers an average rate of recovery k_r without accounting for its possible dependence on the backtracked distance, the DNA sequence, or the external applied force. In addition, the model supposes that every TFIIS molecule in the studied TFIIS-Pol II complexes is active. Thus, the extracted rate k_r is likely a lower bound estimate for the TFIIS-stimulated rate of recovery.

The single-molecule experiments presented here allowed us to separate pauses from active elongation of Pol II. This separation

reveals that the effect of TFIIF is primarily in regulating the pausing phase by decreasing the frequency at which the enzyme enters a pause and by shortening their durations in a force-dependent manner. In the presence of TFIIF, the pauses become shorter under assisting loads (Fig. S1), but they remain unchanged under opposing loads (Fig. 1*F*). It is possible that the high propensity of the enzyme to backtrack under opposing forces partly masks the effect of TFIIF. Using the previously proposed backtracking kinetic model for transcriptional pauses (22, 24, 28, 30, 31), we can express the reduced pause durations in the presence of TFIIF in terms of a reduced backward diffusion rate (k_b), an increased forward diffusion rate (k_f), a combination of both, or an increase in the intrinsic diffusion rate (k_0). Our results do not allow us to discern between these possible scenarios. TFIIF has been shown to help stabilize the short RNA-DNA hybrid during the initiation phase (14). A similar stabilization of the RNA-DNA hybrid during the elongation phase could constitute the mechanism by which TFIIF prevents pause entering and shortens pause durations. Specifically, the TFIIF-enhanced stability of the RNA-DNA hybrid disfavors fraying of the 3' end from the active site of the enzyme; thus, forward translocation is favored over pausing. As Pol II begins to backtrack, the opening of such a hybrid is difficult, which should lead to a decrease in k_{b1} and k_b . Similarly, if the closure of the RNA-DNA hybrid near the active site during backtracking is favored by TFIIF, the k_f would increase. Perhaps, the effect of TFIIF could be equally distributed between all of these processes.

Alternatively, the observation that TFIIF shortens the durations of pauses only under assisting loads could indicate the existence of pauses that are not associated with enzyme backtracking. It has been shown that the bacterial polymerase can exist in a nonbacktracked paused state characterized by an open clamp conformation (39). Because TFIIF has been proposed to stabilize the closed clamp conformation of Pol II (16), it is possible that under an assisting load this factor could both decrease the probability of pausing by preventing entering into an analogous (nonbacktracked) paused state for the eukaryotic enzyme and shorten its lifetime in this state.

As each factor enhances transcription elongation in a different manner, combining both TFIIS and TFIIF could in principle significantly improve the efficiency of transcription elongation both on bare and on nucleosomal DNA as has been described for the human system (27). However, we did not observe significant enhancement in the presence of both TFIIS and TFIIF relative to the factors alone. Because TFIIF reduces the probability of pausing, its presence would be expected to weaken the effect of TFIIS, the activity of which necessarily depends on a backtracked complex. This mechanistic "interference" may explain why the presence of both factors does not result in a quantitatively additive effect. When both factors are present with nucleosomal DNA, we observed a weak enhancement of nucleosomal passage relative to when either factor is present in isolation. Thus, with the yeast system, we do not observe the quantitatively additive effect described in a previous bulk study of human nucleosomal transcription elongation when both TFIIS and TFIIF were present in the medium (27).

In summary, our studies provide a quantitative kinetic model of the mechanism through which transcription factors TFIIS and TFIIF affect the elongation process and highlight the importance of pausing for regulating transcription in the cell. The molecular mechanisms we describe for these transcription factors show that they have evolved to limit the time Pol II remains in a catalytically inactive, paused state. Elucidating the function, at the single-molecule level, of other transcription factors known to play a role in transcription elongation will be of central importance to ultimately understand the detailed mechanisms through which Pol II activity is regulated in eukaryotes.

Methods

Proteins and DNA Preparations. Yeast transcription factor TFIIS ($\Delta 1-113$) with a 6x-His tag was expressed in *Escherichia coli* BL21 (DE3) and purified using a HisTrap HP column (GE Healthcare). The purified protein was dialyzed

against 50 mM NaCl, 50 mM Tris, pH 8.0, and 10% (wt/vol) glycerol for storage. The expression plasmid was a gift from the C. Kane laboratory (Department of Molecular and Cell Biology, University of California, Berkeley, CA). Two recombinant subunits of yeast TFIIF (Tfg1–Tfg2) were expressed and purified as previously reported (40). Biotinylated wild-type RNA polymerase II (*Saccharomyces cerevisiae*, unphosphorylated C-terminal domain) was purified as previously described (21). Transcription elongation complexes (ECs) were prepared as previously described (22, 31). A brief description can be found in *SI Methods*.

Single-Molecule Transcription Assay. The optical-tweezers-based single-molecule transcription elongation assays were performed in passive force mode as previously reported (22, 24, 28, 30, 31). Briefly, opposing force experiments were started below 3 pN and Pol II molecules were considered active only

when they transcribed and reached a force higher than 3.5 pN. Assisting force experiments were carried out in the range 5–8 pN and Pol II molecules were considered active only when they transcribed at least to the nucleosome entry region. For experiments in the presence of transcription factors, 2 μ M of TFIIF and/or 2 μ M of TFIIS was mixed with 1 mM NTP to restart transcription elongation. Data collection and analysis were performed as described elsewhere (30).

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