NTP-driven Translocation by Human RNA Polymerase II*

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We report a “running start, two-bond” protocol to analyze elongation by human RNA polymerase II (RNAP II). In this procedure, the running start allowed us to measure rapid rates of elongation and provided detailed insight into the RNAP II mechanism. Formation of two bonds was tracked to ensure that at least one translocation event was analyzed. By using this method, RNAP II is stalled briefly at a defined template position before restoring the next NTP. Significantly, slow reaction steps are identified both before and after phosphodiester bond synthesis, and both of these steps can be highly dependent on the next templated NTP. The initial and final NTP-driven events, however, are not identical, because the slow step after chemistry, which includes translocation and pyrophosphate release, isregulated differently by elongation factors hepatitis δ antigen and transcription factor IIF. Because recovery from a stall and the processive transition from one bond to the next can be highly NTP-dependent, we conclude that translocation can be driven by the incoming substrate NTP, a model fully consistent with the RNAP II elongation complex structure.

Pre-steady state kinetic analysis allows the progress of an enzymatic reaction to be tracked in real time (1, 2), and coupling enzyme functional dynamics to the structure provides the clearest insight into the mechanism. In this paper, we compare the first transient state kinetic studies of human (Homo sapiens) RNAP II1 to the x-ray structure of the yeast (Saccharomyces cerevisiae) RNAP II elongation complex (EC) (3). These studies give new insight into the RNAP II mechanism and demonstrate the feasibility of a detailed kinetic study of a highly regulated enzyme that is at the hub of gene control in human cells.

There is increasing recognition that transcriptional elongation is highly regulated in eukaryotes (4–8). As an example, hepatitis δ antigen (HDAg) strongly stimulates RNAP II elongation in vitro (6, 9). HDAg is the sole gene product of the small RNA genome of hepatitis δ virus, which is maintained as a satellite particle by hepatitis B virus. The role of HDAg in elongation may be clinically significant because hepatitis δ virus often complicates severe and chronic presentations of human hepatitis B virus infection. The general cellular transcription factor IIF (TFIIF) has been shown to stimulate RNAP II elongation 5–10-fold in vitro, by suppressing transcriptional pausing (10–16). The role of TFIIF in elongation may be of particular importance during the promoter escape phase of the transcription cycle (17, 18). Here viral HDAg and cellular TFIIF are used as probes of H. sapiens RNAP II elongation.

In this work, we use rapid quench kinetics to demonstrate critical NTP-dependent steps during RNA synthesis. First, we analyzed recovery from a stall at a defined template position, in the presence of TFIIF or HDAg. During stall recovery, two fractions of EC were clearly observed on the active pathway, and most significantly, these ECs had different requirements for binding and utilizing the incoming substrate NTP. This observation strongly indicates a substrate NTP-induced fit mechanism, in which the NTP first binds and then helps to convert the EC to a fully active form. Second, in the TFIIF-stimulated mechanism but not in the HDAg-stimulated mechanism, a slow step after phosphodiester bond formation is also highly dependent on the incoming NTP. Thus, in the presence of TFIIF, elongation is NTP-driven at both the beginning and the end of a single bond addition cycle, but only one of these NTPs can be the substrate for phosphodiester bond formation at a single position. The other NTP appears to be the substrate for addition of the subsequent bond. These observations lead to the following conclusions: 1) RNAP II elongates according to a substrate NTP-induced fit mechanism; 2) translocation can be induced by prior NTP binding. Translocation must occur at either the beginning or the end of each bond addition cycle, and in the presence of TFIIF, both are highly dependent on the next templated NTP. Significantly, HDAg eliminates the high NTP dependence of the slow step after phosphodiester bond formation, demonstrating the unusual nature of the RNAP II mechanism in the presence of TFIIF. As with TFIIF, the HDAg-stimulated mechanism shows evidence of substrate NTP-induced fit during escape from a stall, but, unlike TFIIF, NTP dependence is not detected with HDAg in the normal processive transition between bonds.

Comparing the kinetics of RNAP II elongation with the yeast EC structure reverses the view of how NTPs are loaded, alters our understanding of the translocation mechanism, and provides new insight into transcriptional efficiency and fidelity.

EXPERIMENTAL PROCEDURES

Cell Culture, Extracts, and Proteins—HeLa cells were purchased from the National Cell Culture Center (Minneapolis, MN). Extracts of HeLa cell nuclei were prepared as described (19). Recombinant TFIIF (20, 21) and HDAg (9) were prepared as described.

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The abbreviations used are: RNAP II, RNA polymerase II; EC, elongation complex; HDAg, hepatitis δ antigen; TFIIF, transcription factor IIF; DNAP, DNA polymerase.

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The Running Start, Two-bond Elongation Assay—The running start, two-bond elongation assay is shown in Fig. 1 (B, 20). Initiation was from the adenoavirous major late promoter with a modified downstream sequence so that a 40-nucleotide transcript can be synthesized in the absence of ATP and GTP. An extract of human HeLa cells was the source of transcription factors. C40 (a 40-nucleotide RNA ending in a 3' CTP) ECs were synthesized by addition of 10 μM DATP, 300 μM ApC dinucleotide, 5 μM per reaction (0.25 mM CTP, and 20 μM UT) ECs were then washed with 1% Sarkosyl and 0.5 mM KCl to dissociate initiation, elongation, pausing, and termination factors, and then re-equilibrated with transcription buffer. To commence the reaction, C40 ECs were elongated, pausing, and termination factors, and then re-equilibrated and the indicated concentration of GTP. Rate measurements from 0.002 final working NTP concentrations are 5/3M CTP, 5/3M UTP, (functionally saturating amounts) and 20 μM (initial working concentration) CTP and UTP (to maintain ECs at C40). Downstream of C40 the sequence is 40CAAAGG45. On the bench top, an equal sample volume of 200 μM ATP (initial working concentration 100 μM) was added in transcription buffer. The ATP pulse time was 60 s with HDAg, 30 s with TFIIF, and 120 s in the absence of an elongation factor. Times were optimized for each procedure (23). During the pulse, EC samples were injected into the left sample port of the Kintek Rapid Chemical Quench-Flow (RQF-3) instrument and mixed with GTP added from the right sample port. Due to subsequent equal volume mixing events, the final working NTP concentrations are 5 μM CTP, 5 μM UTP, 50 μM ATP and the indicated concentration of CTP (300 μM dATP, 0.25 μM ApC). Two to 4 s were done using the KinTek instrument, and longer time points were done on the bench top, all at 25 °C.

After quenching reactions with 0.5 M EDTA, beads were collected; supernatant was removed, and samples were dissolved in 90% formamide loading dye containing 1% SDS. Samples were boiled for 2 min and RNAs separated in 14–18% polyacrylamide (20:1 acrylamide/bisacrylamide) gels containing 5% w/v urea and 1X Tris borate-EDTA. Gels were analyzed using a Amersham Biosciences PhosphorImager. Each gel lane was analyzed independently for percent of signal present in G44 plus all longer transcripts or G45 plus all longer transcripts compared with A43 plus all longer transcripts. The data were handled in this way to particularly compensate for occasional inconsistency in recovery or loading of samples.

Quality of RNAP II ECs—The complex kinetics we report demonstrate multiple conformers of A43 EC at the time of GTP addition in the running start, two-bond protocol. Because ECs were isolated on bead templates from HeLa nuclear extracts, it is reasonable to consider whether A43 ECs differ in their kinetic properties because of experimental treatments or because of damage to a subset of ECs during preparation. However, A43 conformational states are determined by treatments that occur after EC purification. The initial conformational states detected at A43 are different in the presence of TFIIF, HDAg, or in the absence of an elongation factor, showing that protein factors drive RNAP II between functional modes. Furthermore, increasing GTP concentration blurs the distinction between different kinetic states, indicating that RNAP II changes conformation through interactions with substrate, as expected for an RNAP. Also, the distribution of A43 states is dependent on the time of stalling at A43, demonstrating reversibility between states (23). A43 conformational states, therefore, are selected based on treatments (protein factors, substrate, and time of incubation) after purification and are not an artifact of preparation. In the purification scheme, RNAP II molecules are selected for the ability to initiate transcription accurately in concert with the general initiation factors, and all C40 and A43 complexes are active in elongation. Sarkosyl and salt treatment appears to strip all contaminating transcription factors and replicating activities from the EC (12).

Kinetic Models—Kinetic models were designed based on a qualitative assessment of rate data, as described under “Results,” and model independent analysis of rate data (24, 25) (not shown). The program DY-NAFIT (26), which utilizes non-linear least squares curve fitting to obtain the optimal fit to a kinetic model, was used to estimate rate constants from the rate constants listed in Table I. These rate constants were determined experimentally, so the values reported are meant to represent a simulation of the mechanism with the caveat that future refinement will be necessary to determine fully the accurate rate constant values. The rate constants used for simulations, however, give a reasonable qualitative and quantitative description of the rate equation. These rate constants apply fit the primary characteristics of the data sets, whereas alternative schemes prove inadequate to model the data. Furthermore, the models we espouse seem consistent with the S. cerevisiae RNAP II EC structure (3).

The kinetic pathway shown in Fig. 4A is the simplest induced fit mechanism with a pausing pathway that allows access of the active site after an NTP-induced fit conformational change (see under “Results” and “Discussion”). This is an adequate kinetic model for RNAP II elongation stimulated by HDAg. To fit the HDAg-stimulated mechanism requires a minimum of three initial states at A43 as follows: A43a (23% of total ECs); A43b (27% of total ECs); and A43c (50% of total ECs). From model-independent analysis (not shown), the fastest rate is estimated as 1250 s−1, and the fastest rate on the slow pathway (A43a-GTP → A43b-GTP)-A43b-GTP) is 20 s−1. A slow step after chemistry is required to fit the sigmoidal shape of G45 rate curves (Figs. 3B and 4A). The rate of this slow step is well determined as 15.5 ± 0.5 s−1.

The pathway shown in Fig. 4B is the simplest induced fit model (including a pausing pathway), with an open active site, that also correctly accounts for both the slowest elongation factor (S. cerevisiae RNAP II ECs) and the fastest rate on the slow pathway (TFIIF- and HDAg-stimulated model of the G44 bond addition cycle (see under “Results” and “Discussion”). This mechanism is adequate to model rates of elongation stimulated by TFIIF through formation of two phosphodiester bonds. The simple induced fit model (Fig. 4A) is adequate to fit the TFIIF data set through formation of the G44 phosphodiester bond (not shown) but not through formation of the G45 bond. In the presence of TFIIF, a minimum of three initial conformers at A43 are required: A43a (55% of total); A43b (10% of total); and A43c (35% of total). Model independent kinetic analysis indicates that the fastest pathway for G44 synthesis is 1,800 s−1 (not shown) and is estimated as 1200 s−1 in the model shown in Fig. 4B. The model independent analysis estimates the slower pathway from A43a as >100 s−1 (not shown). Furthermore, there is a requirement for loading two substrate GTPs in a single G44 bond addition cycle. The GTP requirement is fulfilled by loading both the n + 1 and n + 2 GTPs to A43a before translocation. As expected from the S. cerevisiae RNAP II EC structure, GTPs held primarily by base pairing are bound weakly (GTP off-rates of 10,000 s−1), the DYNAFIT program would select faster dissociation rates. Elongation rate data in the presence of TFIIF (Figs. 3D and 4B) require a fast elongation pathway that dominates at high GTP concentrations and a slow pathway that dominates at limiting GTP concentrations.

Residuals (a statistical test; Fig. 3) indicate that these models converge to experimental data generally within 5% or 10%, indicating the robustness of the simulation fits. A very slight improvement in the models is the simplest induced fit model (including a pausing pathway), with an open active site, that also correctly accounts for both A43 ECs proved unsuitable for measuring the most rapid elongation rates through formation of multiple bonds. Precisely stalled RNAP II ECs, immobilized on magnetic beads, were initiated from the adenovirus major late promoter and isolated with Sarkosyl and salt washing (Fig. 1). C40 ECs contain a 40-nucleotide, 32P-labeled RNA, ending in a 3′-CMP base. The sequence downstream of C40 is 40CAAAGG45. Because C40 ECs proved unsuitable for measuring the most rapid elongation rates, a running start, two-bond protocol was adopted. In the presence of 20 μM CTP and UTP, 100 μM ATP was added to advance C40 ECs to the A43 position. After a brief stall at A43, a steady state distribution was established between paused and active A43 ECs (23), such that, when GTP was added, rapidly rates for G44 and G45 synthesis could be estimated reproducibly. In this experimental design, G44 synthesis rates reflect recovery from a stall at A43, and initial G45 synthesis rates reflect processive elongation from G44 → G45, including a translocation of the RNA-DNA hybrid and template DNA.

Because the running start assay allowed us to commit a significant fraction of A43 ECs to rapid elongation, we adapted this method to analyze GTP-dependent steps along the forward
synthesis path. In Fig. 2, we compare synthesis through the G44 and G45 positions in the presence of the RNAP II elongation factors HDAg and TFIIF. After a brief ATP pulse, GTP was added at the indicated concentrations, and reactions were quenched at various times. The protocol is indicated in Figs. 1 and 2A. The ATP pulse time, optimized for each reaction, is 120 s in the absence of a stimulatory factor (Fig. 2B), 60 s in the presence of HDAg (Fig. 2C), and 30 s in the presence of TFIIF (Fig. 2D). In Fig. 2B, elongation is shown in the absence of a stimulatory factor to confirm that TFIIF and HDAg enhance elongation in the running start assay (compare Fig. 2, B–D). Rates of G44 and G45 synthesis were evaluated in terms of the percent of transcript at G44 or G45 plus all longer transcripts. In this way, rates of disappearance of G44 and G45 could be neglected.

The running start, two-bond protocol reveals significant details of the RNAP II elongation mechanism. In the presence of HDAg or TFIIF, synthesis rates for G44 differ from synthesis rates for G45, demonstrating the importance of monitoring two bonds. With the running start method, analysis of the G44 bond is expected to provide detail about RNAP II conformational states and kinetic intermediates and any effects of recovering from the 30- to 120-s stall at A43. Rates of G45 synthesis, on the other hand, are expected to reveal characteristics of the approach to processive elongation. The rates of first G45 appearance should provide insight into translocation, because translocation and pyrophosphate release must occur between synthesis of the G44 and G45 bonds. If only a single bond is tracked, information about translocation could be lost, because the state(s) of translocation prior to addition of substrate cannot be known.

Two Intermediates on the Forward Elongation Pathway with Distinct GTP Dependence—In Fig. 3, complete data sets are shown for elongation through the G44 and G45 positions, in the presence of HDAg (Fig. 3, A and B) or TFIIF (Fig. 3, C and D). The comparison between the HDAg- and TFIIF-stimulated data sets provides clear insight into many details of the RNAP II mechanism and its regulation by elongation factors. Curve fits are derived from the kinetic mechanisms shown in Fig. 4, A and B. Residuals shown below each plot in Fig. 3 demonstrate how closely experimental data can be fit with these simulations.

Analysis of the rates of G44 synthesis (Fig. 3, A and C) indicates that RNAP II must utilize an induced fit mechanism, in which binding substrate GTP modifies the A43 EC conformationally to become catalytically competent. Notably, during the 60- or 30-s stall at A43, delayed ECs fractionate between at least three different A43 states, two of which are evident on the forward synthesis pathway and one of which is strongly paused. Furthermore, two A43 ECs are observed on the active synthesis pathway with differing dependence on the concentration of the incoming GTP substrate.

In the presence of HDAg, ECs partition between three states (Fig. 3A). Two A43 ECs are found on the active synthesis pathway, but these two EC conformers have different responsiveness to GTP. About 27% of A43 ECs are in the most highly poised state (Fig. 3A, fraction b; 0–27% of total ECs; \( k_{\text{obs,fast}} = \frac{1250}{} \text{s}^{-1} \) (see under “Experimental Procedures”). An additional 23% of A43 ECs are more dependent on GTP substrate to advance rapidly (Fig. 3A, fraction a; 27–50% of total ECs; \( k_{\text{obs,slow}} = \frac{20}{} \text{s}^{-1} \) (see under “Experimental Procedures”). Because two classes of A43 EC are detected with different responsiveness to GTP concentration, this is evidence of induced fit in the RNAP II mechanism. GTP binds to a less highly poised EC (fraction a) and converts it to a more highly poised EC (fraction b). In the presence of HDAg, 50% of A43 ECs are initially paused (Fig. 3A; 50–100% of total ECs; \( k_{\text{obs,slow}} = \frac{0.05}{} \text{s}^{-1} \)).

In the presence of TFIIF, three classes of A43 EC are detected but with different occupancy than those observed with HDAg (compare Fig. 3, A and C). Two of these classes of A43 EC are on the active synthesis pathway, and one is paused. About
10% of A43 ECs elongate rapidly to the G44 position even at GTP concentrations that are too low to support subsequent rapid elongation from G44 to G45 (Fig. 3C, fraction b, 0–10% of total ECs; \(k_{\text{obs,fast}} > 500\) s\(^{-1}\)). These A43 ECs are highly poised to bind GTP substrate and incorporate GMP. A distinct fraction of A43 EC (about 55% of total) elongates rapidly at high GTP concentrations but much more slowly at low GTP concentrations (Fig. 3C, fraction a; 10–65% of total ECs; \(k_{\text{obs,slow}} > 100\) s\(^{-1}\)). This fraction of A43 EC (fraction a) requires prior GTP binding to convert to a catalytically competent state (fraction b), consistent with a substrate GTP-induced fit mechanism for RNAP II elongation. The remaining 35% of A43 ECs (Fig. 3C; 65–100% of total ECs; \(k_{\text{forward}} \approx 0.09\) s\(^{-1}\)) are strongly paused but eventually can be extended (Fig. 2D). Because multiple A43 ECs are detected that respond differently to substrate GTP concentrations, with both HDag and TFIIF, the RNAP II EC assumes conformations that must first bind GTP and then be converted to a form capable of catalyzing phosphodiester bond formation.

**Regulation of First Appearance of G45 by Substrate GTP**—Judging from the initial times of G44 and G45 appearance on gels (Fig. 2, B–D), the most rapid rates of G44 synthesis must be 5–10-fold faster than the rate of initial G45 appearance, which is surprisingly slow. If this were not the case, G45 would be detected by the 0.002–0.005-s time points, but G45 is not detected until 0.02–0.05 s, even at high GTP concentration. This conclusion is further demonstrated by quantitation of gel data (Fig. 3, B and D). Analysis of G45 synthesis rate curves shows that the initially slow appearance of G45 can be attributed to a slow conformational step after G44 phosphodiester bond formation. This conclusion is demonstrated by the sigmoidal shapes of the rate curves shown in Fig. 3, B and D. The distinctive shapes of these curves can only be described by a slow, normally irreversible step in the RNAP II elongation mechanism after G44 phosphodiester bond synthesis but before rapid G45 synthesis can commence (see Fig. 4, A and B). Note that the interval in which this slow conformational step occurs must include the translocation event between G44 and G45 synthesis.

In the presence of HDag (Fig. 3B), there is a slow step after G44 phosphodiester bond formation that accounts for the slow first appearance of G45. This slow step is indicated by the sigmoidal shapes of G45 synthesis rate curves. Notice that the sigmoidal rate curves all approach the time axis at a similar intersection point (Fig. 3B, open arrow). This result shows that, in the presence of HDag, the lag in G45 first appearance is not highly dependent on GTP concentration, although in the presence of TFIIF (Fig. 3D), the lag duration is highly GTP-dependent. For the HDag-stimulated mechanism, the slow step after chemistry can be modeled by a first order rate constant, lacking GTP dependence, of 15.5 ± 0.5 s\(^{-1}\) (Fig. 4A).

Surprisingly, in the presence of TFIIF, the situation is very different. At 250 and 100 μM GTP, G45 appears in an apparent burst (Fig. 3D), but these rate curves are sigmoidal when plotted with an expanded time axis, indicating the slow step after chemistry (lag of 0.02–0.05 s (Fig. 2D)). From 10 to 25 μM GTP, G45 synthesis rate curves are notably sigmoidal in shape, further demonstrating the slow step after G44 bond formation. Surprisingly, at 1 and 2 μM GTP, almost no G45 synthesis is observed within 5 s, although eventually these ECs will advance (data not shown). This result demonstrates the extreme GTP dependence of this slow step in the TFIIF-stimulated mechanism. So, after a stall at A43 in the presence of TFIIF, both the beginning phase and the ending phase of the G44 bond addition cycle are highly dependent on the next incoming GTP substrates (Fig. 3, C and D). In the running start protocol, this
unusual condition arises because elongation was stalled at the A43 position. Because the GTP-driven event at the end of the G44 bond addition cycle occurs after chemistry, this event cannot be attributed to utilization of GTP as a substrate for G44 bond formation and primarily reflects entry of the GTP substrate for G45 synthesis.

As with TFIIF, HDAg-mediated recovery from a stall at A43 is GTP-dependent (Fig. 3A), demonstrating the GTP-induced fit mechanism. With HDAg, however, the transition between synthesis of the G44 and G45 bonds is not noticeably dependent on the incoming substrate GTP (Fig. 3B), as it is in the presence of TFIIF (Fig. 3D). Therefore, HDAg and TFIIF regulate the normal processive transition between bonds (translocation) in distinct ways. HDAg simplifies this step and makes it less dependent on GTP. Also, recovery from a stall is found to be a distinct process from the normal processive transition between bonds, because TFIIF and HDAg regulate these steps differently. One result of HDAg-mediated stimulation is that elongation is facilitated at GTP concentrations that are very restrictive in the presence of TFIIF, as if HDAg facilitates a rate-limiting step (translocation) in the RNAP II mechanism (compare Fig. 3, A–D).

Kinetic Modeling—RNAP II appears to extend an RNA chain according to an induced fit mechanism, in which the active site remains available for substrate NTP binding, even when the conformational change precedes substrate loading. Furthermore, RNAP II establishes a steady state condition between the pausing and active synthesis pathways that is maintained for minutes at the A43 position (23). The simplest kinetic model that will satisfy these conditions is shown in Fig. 4A. Induced fit requires that substrate NTP normally binds prior to a conformational step in the mechanism (27–29). In this paper, we argue strongly for an induced fit mechanism of RNAP II elongation. Specifically, we argue for substrate NTP-driven translocation of the RNA-DNA hybrid past the RNAP II active site, which is a version of an induced fit mechanism, in which translocation is the NTP-directed conformational change. We present our argument assuming that A43a and A43b states are natural intermediates on the RNAP II elongation pathway. Formally, however, A43a and A43b could be on separate pathways. For instance, A43a and A43b could represent distinct RNAP II ECs with different activities (i.e. because of differential protein composition or covalent modification). We find this alternate view untenable for the following reasons: 1) relative occupancy of A43a and A43b depends on whether TFIIF or HDAg is added to the reaction, showing that these states are inter-convertible (Figs. 3 and 4); and 2) fast and slow complexes are detected for G44 synthesis, after the stall at A43, but not for G45 synthesis (Fig. 3), so intrinsically fast and slow ECs are not indicated. Further discussion of this issue is found under “Experimental Procedures.”

Experimental data, therefore, require two A43 states, designated A43a and A43b, that are occupied to different extents in the presence of HDAg or TFIIF. The conversion of A43a → A43b is a conformational change that may equate with the translocation step. A43a requires prior GTP binding to make this conformational transition rapidly; otherwise, the transition is slow. A43b is more highly poised on the forward synthesis pathway and apparently has already undergone this conformational transition but is yet capable of binding GTP and engaging in chemistry. Both the A43a and A43b conformational states are therefore available to bind GTP in the pro-
posed mechanism. The simple induced fit mechanism (see Fig. 4A) will account very well for rates of G44 and G45 synthesis by RNAP II stimulated by HDAg (Fig. 3, A and B). The simple induced fit mechanism will account for G44 synthesis rates by TFIIF (not shown) but not for the rates of G45 synthesis, which requires GTP dependence at both the beginning and end of the G44 bond addition cycle.

The TFIIF-stimulated mechanism, therefore, was fit using the more complex kinetic model shown in Fig. 4B. This is the simplest induced fit mechanism that can account for GTP dependence at both the beginning and end of the G44 bond addition cycle. For the TFIIF-stimulated mechanism, a kinetic scheme is shown for the formation of two bonds. The scheme used to fit synthesis of the G45 bond is a subset of the mechanism used to fit G44, eliminating steps that are peripheral and therefore unnecessary for modeling. We assume that the mechanisms for G44 and G45 synthesis include all of the same steps and utilize similar rate constants, but in the running start protocol, many steps observed for G44 synthesis are largely undetected in G45 synthesis. For instance, the pausing pathway is observed in G44 synthesis, because of the stall at A43, but pausing is not observed in G45 synthesis, unless the EC is delayed at G44 (i.e. by GTP limitation). Effectively, RNAP II tends to bypass both the pausing pathway and perhaps the G44a conformational state during rapid elongation. Some of the rate constants for G45 synthesis appear different from those for G44 synthesis. This is attributable to the simplified mechanism used to model formation of the G45 bond.

The only difference between the mechanisms in Fig. 4, A and B, is the involvement of the n + 2 GTP substrate (n = RNA length) in the slow transition from G44 → G45 synthesis. In the mechanism shown in Fig. 4B, both the n + 1 GTP and the n + 2 GTP pre-load and then, in turn, drive translocation of the RNA-DNA hybrid past the RNAP II active site, establishing the conditions for chemistry. Consistent with the model in Fig. 4B, the data in Fig. 3D demand two forward pathways for chemistry in the presence of TFIIF: a faster pathway supported by higher GTP concentrations and a slower pathway that predominates at limiting GTP concentrations. The HDAd-stimulated rate data only require a single pathway after chemistry for data fitting. Although our model in Fig. 4B recapitulates the general shapes of rate curves, the lags observed in the initial appearance of G45 are more highly GTP-dependent than we can describe with this kinetic model. This result means that the TFIIF-stimulated mechanism is more highly GTP-dependent, at this slow, regulated step from G44 → G45 (translocation), than can be accounted for by the simulation shown in Fig. 4B, a model that confers high GTP dependence. Therefore, in the presence of TFIIF, translocation of the RNA-DNA hybrid at this position is a slow step in the mechanism that is highly dependent on the incoming substrate GTP.

In Fig. 4C a popular model for elongation, which we refer to as an “equilibrium sliding” model, is shown. In such a model, there is a rapid equilibrium between the pre- and post-translocated states, and NTP substrate binding stabilizes the post-translocated EC (30–39). Our kinetic data argue strongly against the equilibrium sliding model for H. sapiens RNAP II. A43a and A43b cannot be connected by a rapid equilibrium, because escape from A43a is more highly GTP-dependent than escape from A43b. Therefore, there must be distinct GTP-dependent pathways forward from A43a and A43b and interconversion between A43a and A43b must be relatively slow, as indicated in our simulations (Fig. 4, A and B). Our data clearly indicate that, in the presence of TFIIF, translocation is GTP-driven in the A43a → G44 and G44 → G45 bond addition cycles, and these experimental observations cannot be reconciled with an equilibrium sliding model.

**DISCUSSION**

**Translocation Can Be Driven by the Incoming Substrate NTP**—We have tracked the progress of kinetically homogeneous populations of human RNAP II molecules through the sequence 40CAAAGG45, concentrating on synthesis of the G44 and G45 bonds. After a brief stall at A43, the beginning of the G44 bond addition cycle is dependent on the incoming substrate GTP for a significant fraction of ECs (A43a fraction). After G44 chemistry, the final phase of the G44 bond addition cycle can also depend on the next incoming substrate GTP. Because the transition from G44 → G45 requires translocation, movement of the RNA-DNA hybrid relative to the RNAP II active site must occur during this GTP-driven phase of the bond addition cycle. By stalling the EC at A43, however, it appears that we have established a situation in which formation of G44 can include two GTP-driven translocations. The first translocation is driven by the incoming substrate GTP for G44 synthesis, and the second translocation is driven by the incoming GTP substrate for G45 synthesis. That both the early and ending phases of the G44 bond addition cycle are GTP-dependent, in the TFIIF-stimulated reaction, fully establishes that translocation can depend on a GTP-driven conformational change in the RNAP II mechanism. We suggest that the GTP-induced step is translocation, rather than another obligatory but unknown conformational change that must precede or follow translocation. Recovery from a stall at A43 and processive elongation from G44 → G45 are likely to be different processes, because the G44 → G45 transition requires translocation and pyrophosphate release. Recovery from a stall at A43 is likely to require translocation for a fraction of ECs but not pyrophosphate release, which is completed during the stall. Furthermore, these steps are regulated differently by TFIIF and HDAd.

Dwelling at A43 in the absence of GTP appears to reverse the translocation step for a significant fraction of A43 ECs (designated A43a). In the presence of HDAd, 23% of A43 ECs are detected that are on the pathway for synthesis but require a higher concentration of GTP to progress rapidly (Fig. 3A, fraction a). Another 27% of A43 ECs are more highly poised on the active synthesis pathway (Fig. 3A, fraction b). In the presence of TFIIF, 55% of ECs are on the pathway for rapid synthesis but require a higher GTP concentration for activation, whereas another 10% of A43 ECs are more highly poised for synthesis (Fig. 3C, fractions a and b). By assuming that both A43a and A43b conformers are natural intermediates on the elongation path, the A43b EC is more highly poised for synthesis than the A43a EC, and the A43b EC appears to be less dependent on the concentration of GTP substrate for elongation. Furthermore, because both classes of A43 EC bind GTP and engage in G44 synthesis, the RNAP II active site must remain open to substrate binding after the GTP-driven conformational change. This distinction is important because the dNTP-induced fit conformational change postulated for elongation by single-subunit DNAps appears to involve rotation of a critical α-helix to partially close the catalytic site to dNTP binding (see Refs. 25, 27–29, 40–43). In the RNAP II NTP-induced fit mechanism, the active site remains open to substrate binding.

**Regulation by HDAd and TFIIF**—To account for differences in HDAd- and TFIIF-regulated elongation, we offer the following explanation. We suggest that TFIIF supports a tight RNAP II clamp holding the RNA-DNA hybrid and an optimum geometry of the RNAP II active site for catalysis. Because the clamp is tight, the RNAP II bridge α-helix is wedged against the RNA-DNA hybrid (see below). In such a case, when elongation
is stalled, the EC reverts primarily to the pre-translocated state, and the next templated NTP substrate is required to drive translocation, as observed in our experiment. This idea is consistent with our observation that the A43a (pre-translocated) EC has 55% occupancy in the presence of TFIIF and only 23% occupancy in the presence of HDAG (Figs. 3 and 4). We believe that HDAG may loosen the RNAP II clamp to facilitate RNA-DNA hybrid translocation. In such a case, elongation becomes less dependent on the prior loading of the next templated NTP because the incoming NTP is no longer required to force the RNA-DNA hybrid away from the bridge α-helix (see below). We do not believe that the RNAP II mechanism is truly different in the presence of HDAG and TFIIF. Rather, in the presence of HDAG, the model appears simpler because loosening of the RNAP II clamp stimulates translocation and accelerates the slow elongation pathway, so that it can no longer be distinguished from the rapid pathway. In support of this idea, in both mechanisms, NTP substrate-induced fit is observed for G44 synthesis, and a slow step is observed after G44 chemistry that precedes the first appearance of G45. Therefore, the basic RNAP II mechanism is similar in the presence of TFIIF or HDAG. In the HDAG-stimulated mechanism, a downside to relieving tension on the RNAP II clamp may be that the geometry of the active site becomes less optimal for chemistry, decreasing the fastest elongation rates, and fidelity may be sacrificed. In hepatitis δ virus RNA replication and transcription, HDAG is thought to convert *H. sapiens* RNAP II from a DNA template-dependent RNAP to an RNA template-dependent RNAP (9, 44–47), and loosening the RNAP II clamp may facilitate this loss of fidelity in template selection.

**Substrate NTP-driven Translocation and Allostery**—Recently, Erie and colleagues (24) reported transient state kinetic studies of *E. coli* RNAP elongation. They proposed an allosteric model to describe slow rates of RNA synthesis at low substrate NTP concentrations. We concur with their observation that elongation rates are unexpectedly slow when substrate NTP concentrations fall below a threshold value. Because the *E. coli* RNAP allosteric site appears to be template-specified in their study, it is difficult to distinguish the allosteric site from the substrate site, unless the allosteric conformational change is translocated, as we suggest here. In such a case, the “allosteric” site becomes the substrate site. Our data strongly indicate that, at limiting substrate NTP concentrations, active site translocation is the critical, rate-limiting step along the RNA synthesis pathway, and translocation is induced by prior NTP binding. In our model, only template-specified NTP-loading sites at positions n + 1 and n + 2 (n = RNA length) are required.

To analyze human RNAP II, we were forced to adopt the running start, two-bond protocol to observe rapid elongation rates and to ensure that we would observe at least one translocation event. In studies of *E. coli* RNAP, however, elongation was from ECs that were stalled for a longer time, and in those studies, only a single bond was monitored. In Foster et al. (24), *E. coli* RNAP rate curves have approximately hyperbolic shapes, unlike the biphasic curves observed for *H. sapiens* RNAP II in our study. In more recent work, however, Erie and colleagues have produced rate data at new template positions that produce notably biphasic rate curves. Although *E. coli* RNAP and *H. sapiens* RNAP II both can yield rate curves with biphasic shapes, there are significant qualitative differences between the data sets produced in the bacterial and human systems. Notably, in the bacterial system, the amplitude of the fast phase is more highly dependent on the NTP substrate concentration, consistent with a single primary starting conformation of RNAP at the time of NTP addition and perhaps consistent with the allosteric mechanism from Erie and co-worker (24). In our data set, biphasic rate curves appear to reflect two distinct EC conformations on the active synthesis pathway (A43a and A43b), and the amplitude of the fast phase in our rate curves does not appear to be so highly dependent on NTP concentration, as if the biphasic rate curves may have distinct meanings in the *E. coli* and *H. sapiens* systems. It appears to us that, although homologous, *E. coli* and *H. sapiens* RNAPs may have distinct features in the details of their catalytic mechanisms, and that many more experiments in both systems will be required to fully understand the functioning and regulation of these dynamic molecular machines. For instance, *E. coli* RNAP may utilize an allosteric mechanism, whereas *H. sapiens* RNAP II does not appear to require allosteric control, unless the substrate NTP-induced translocation mechanism that we propose can be described as “allostery.” Generally, allostery requires distinct binding sites for substrate and effector.

*The Substrate NTP-induced Translocation Model*—Our kinetic model indicates that incoming NTP substrates pair to their cognate DNA bases before the resulting base pair is translocated into the RNAP II active site. We therefore inspected the *S. cerevisiae* RNAP II EC structure (3) to determine whether such an NTP loading mechanism is likely or possible. In Fig. 5, we show how substrate NTPs can pre-load through the RNAP II main channel to induce translocation. In the *S. cerevisiae* RNAP II EC structure, the n + 1, n + 2, and n + 3 DNA template bases are single-stranded and apparently accessible to base pair with incoming NTPs loaded through the main channel (Fig. 5A) (3). The non-template DNA strand is disordered in the structure and is therefore unlikely to conflict with NTP loading by this route. Darst and colleagues (48), Kornberg and colleagues (3, 50), and Yokoyama and colleagues (49) have suggested that NTPs might load into the active site through the secondary pore, from the opposite direction than that we propose. In the RNAP II mechanism, however, observation of induced fit is evidence for loading the n + 1 NTP before a conformational change that precedes chemistry. Furthermore, in the TFIIF-stimulated mechanism, the final stage of the G44 bond addition cycle is also GTP-driven (Fig. 3, D and B). Therefore, both the n + 1 and the n + 2 GTPs appear to be loaded prior to rate-limiting conformational steps, occurring both before and after G44 synthesis.

An advantage to tracking two bonds is that at least one of the two NTP-driven steps we observe in G44 synthesis, specifically the step from G44 → G45, must include a translocation. If either the n + 1 or n + 2 GTPs pair to template prior to translocation, these bases must load through the main channel rather than through the secondary pore, because otherwise base pairing is impossible. In the *S. cerevisiae* RNAP II EC structure, before translocation, the n + 1 DNA base is oriented toward the main enzyme channel and is not accessible to the secondary pore. We believe that the secondary pore is normally the route for pyrophosphate release and for release of improperly loaded NTPs that are transferred into the active site but cannot subsequently be incorporated into RNA. We suggest the following: 1) substrate NTPs normally enter the active site through the main channel, in much the same direction of flow as downstream template DNA; and 2) substrate NTPs are continuously pre-loaded in bunches of two or three (Fig. 5). Continuous maintenance of two or three paired NTPs supports efficiency and fidelity of NMP incorporation, because accurate base pairing is confirmed and re-confirmed before loading of a base pair into the active site. No structural barrier is apparent.

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8 D. Erie, personal communication.
to loading the $n + 1$, $n + 2$, and $n + 3$ NTPs through the main channel or to pair them to their DNA cognate bases (Fig. 5A). In the *S. cerevisiae* RNAP II EC structure (3), the only observed barrier to moving the $n + 1$ NTP-DNA base pair from the main channel into the catalytic center is the $n$ base pair (Fig. 5, A and B, gold), and the barrier is removed when the RNA-DNA hy-
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brid translocates (Fig. 5, C and D). Once the n base pair is driven into the post-translocated position, a pore opens for loading the n + 1 NTP-DNA base pair. We detect no steric or electrostatic barriers to loading the triphosphate of the n + 1 NTP through the induced pore, and the amino acid residues that constitute the pore are conserved in multisubunit RNAps from bacteria to man (50).

As noted by Kornberg and colleagues (3, 50), in structures of bacterial Thermus aquaticus RNAP, the bridge α-helix (also called the F helix) may be bent because of contact with the G-loop or “trigger” loop protruding from below (48, 49, 51). Binding of the bridge α-helix in the T. aquaticus RNAP structure prompted Gnatt et al. (3) to propose that bending of the bridge helix might be part of the translocation mechanism. Although structural and cross-linking studies indicate that the bridge helix is capable of flexure (3, 48, 49, 51), bending of the bridge helix may not be necessary during each translocation cycle. As indicated in Fig. 5, passage of the n + 1 NTP triphosphate over the bridge α-helix may induce movement of the n base pair past the active site Mg2+ and rotate the n + 1 base pair into the active site for phosphodiester bond formation, without bridge helix bending. The most extreme conformation of the bridge helix, seen in T. aquaticus RNAP structures (48, 52), will not easily accommodate our model for NTP loading, but conformations in the S. cerevisiae RNAP II and Thermus thermophilus RNAP structures appear completely consistent with our model (3, 49). The most strongly bent configurations of the bridge helix are expected to block movement of the n + 1 DNA base into position, because bending the helix would rotate S. cerevisiae Rpb1 Lys830 (H. sapiens Lys853, E. coli β’ Lys789) into the space through which the DNA base must pass. Thus, the original suggestion that NTPs load through the secondary pore was made based on a structure with a severely bent bridge helix that may be incompatible with ongoing transcription (48, 52).

Modeling NTP loading indicates the importance of conserved S. cerevisiae Rpb1 bridge helix residues Ile825_THr831, particularly S. cerevisiae Rpb1 Ala828 and Thr831 (H. sapiens Rpb1 Ala851 and Thr854, E. coli β’ Ala787 and Thr790), which protrude most directly into the space through which the triphosphate of the incoming NTP must pass. Conserved charged residues, S. cerevisiae Asp826 and Lys830, point down from a straight bridge helix (in the view shown), away from the proposed NTP loading pore, and are not expected to complicate or facilitate triphosphate passage, unless the bridge helix bends. A conserved arginine residue, S. cerevisiae Rpb2 Arg512 (H. sapiens Rpb2 Arg499, E. coli β Arg446) may play a “gatekeeper” role for NTP loading through the main channel. S. cerevisiae Rpb2 Arg712 appears to be positioned to interact with the triphosphate of the n + 1 NTP (Fig. 5B) and then switch, in turn, to contact the triphosphate of the incoming n + 2 NTP, as the n + 1 NTP moves over the bridge helix and the n + 2 NTP makes its approach (Fig. 5, C and D).

It appears that stalled RNAP II ECs may tend to revert to the pre-translocated state, as observed previously with T7 RNAP (53). Inspection of the S. cerevisiae RNAP II EC structure indicates that the RNA-DNA hybrid might be spring-loaded against the bridge α-helix as the RNA-DNA hybrid is pressed by the flexible RNAP II clamp (3). Furthermore, the stalled S. cerevisiae RNAP II EC is found primarily in the pre-translocated form with the active site Mg2+-A atom poised over the previously formed phosphodiester bond. In our kinetic analysis, a large fraction of A43 complexes initially appear to be in the pre-translocated A43a state, even though an adequate kinetic model suggests that elongation passed through the post-translocated A43b state to access the A43a state (Fig. 4, A and B). The n + 1 DNA base is rotated about 90° away from the active site Mg2+ atom. Therefore, catalysis requires that the n + 1 DNA base first swing into position between the RNA-DNA hybrid and the highly conserved bridge α-helix. Our kinetic data support the idea that the incoming NTP normally pairs with its cognate DNA template base prior to translocation of the RNA-DNA hybrid. Formation of the n + 1 base pair, therefore, is expected to facilitate its rotation into the active site pocket. We suggest that base pairing and movement of the n + 1 NTP over the bridge α-helix provides a major driving force to induce translocation of the RNA-DNA hybrid during rapid catalysis.

NTP-induced Translocation Enhances Fidelity—We argue above that the unusual dependence of H. sapiens RNAP II on substrate NTP concentration should perhaps not be attributed to allostery but rather to NTP-induced translocation of the RNAP II active site. But, if our view is correct, why then is translocation dependent on prior binding of the next templated substrate NTP? The images we present in Fig. 5 indicate an assembly line model in which templated NTPs pre-load up to three at a time, and translocation is continually driven by the incoming n + 1 substrate NTP. With each translocation event, a DNA base becomes single-stranded and is expected to pair to its cognate NTP. In addition to the efficiency of the molecular assembly line we describe, we propose that NTP-induced translocation is a mechanism for maintaining fidelity of NMP incorporation during RNA synthesis.

Fidelity is the selection of the specified, templated n + 1 NTP rather than any of the three incorrect choices. We argue that NTP-induced translocation renders mis-incorporation unlikely because the n + 1 base pair must be maintained throughout its long passage over the bridge α-helix (Fig. 5, A–D). To incorporate AMP for GMP, for instance, would require maintenance of an unstable dC-ATP base pair throughout movement into the active site. Most likely, substrate NTP-induced translocation developed as a mechanism to ensure fidelity of RNA synthesis, because this mechanism increases efficiency and accuracy by allowing pre-loading and pre-aligning of multiple NTPs. By using this mechanism, fidelity can be maintained for ATP, GTP, CTP, and UTP substrates without strict chemical recognition of individual bases in the RNAP II active site, which would compromise the flexibility that RNAPs require to utilize four chemically distinct substrates. Very tight binding of substrate is not required for specificity by RNAPs because recognition of each NTP substrate is driven primarily by accurate base pairing, through the NTP-induced translocation mechanism. Incorrectly selected n + 1 NTPs do not remain paired long enough to support translocation, while correctly pairing the specified n + 1 NTP forces the RNAP into the only conformation capable of forming a phosphodiester bond. Adequate kinetic models indicate that GTP affinity may vary significantly over the course of the reaction (Fig. 4, A and B). In the TFIIH-stimulated reaction, modeling indicates that GTP affinity might be highest (estimated as Ka ~6 µM) to the fully translocated substrate-binding site, as expected from the RNAP II EC structure and the NTP-induced translocation model, in which NTPs initially load primarily by base pairing before loading into the RNAP II active site. Because polymerases utilize four chemically distinct substrates, DNAPs and RNAPs are characterized by reaction mechanisms that maintain high fidelity with relatively low substrate affinity. The NTP-induced translocation model shows one way this biological requirement can be achieved.

DNAP and RNAP Mechanisms—In the mechanisms of simple DNAPs, induced fit has been interpreted as a dNTP-induced structural change preceding chemistry, to convert the
EC from a “relaxed” to a “taut” conformation, also referred to as conformational coupling or induced fit (22, 25, 27–29). This change is proposed to enhance fidelity, because a dNTP is mis-loaded, the conformational change is blocked, and the mis-paired substrate can be dissociated before mis-incorporation occurs. In this paper, we argue that H. sapiens RNAP II utilizes a substrate NTP-induced fit mechanism that involves NTP-driven translocation of the RNA-DNA hybrid past the RNAP II active site. If this is the case, a taut conformational change likely. For instance, because translocation by RNAP II active site. Our mechanism, however, does not preclude a relaxed to taut conformational change that tightens the active site just prior to chemistry, and we deem such a conformational change likely. For instance, because translocation by RNAP II involves a slow, substrate NTP-induced conformational change, this implies that loosening of the RNAP II clamp may accompany NTP-driven translocation. If this is the case, a relaxed to taut conversion may accompany RNA II pyrophosphate release may follow and depend upon a RNAP II relaxed conformational change after chemistry.

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REFERENCES
1. Johnson, K. A. (1992) Enzymes 20, 1–61
2. Johnson, K. A. (1995) Methods Enzymol. 249, 38—61
3. Gnaat, A. L., Cramer, P., Fu, J., Bushnell, D. A., and Kornberg, R. D. (2001) Science 292, 1876–1882
4. Conaway, J. W., Shilatifard, A., Dvir, A., and Conaway, R. C. (2000) Trends Biochem. Sci. 25, 375–380
5. Shilatifard, A. (1998) FASEB J. 12, 1437–1446
6. Yamaguchi, Y., Delehousse, S., and Handa, H. (2002) Microbes Infect. 4, 1169–1175
7. Kim, D. K., Yamaguchi, Y., Wada, T., and Handa, H. (2001) Mol. Cells 11, 267–274
8. Krogan, N. J., Kim, M., Ahn, S. H., Zhong, G., Kebor, M. S., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S., and Greenblatt, J. F. (2002) Mol. Cell. Biol. 22, 6975–6992
9. Yamaguchi, Y., Filipovska, J., Yano, K., Furuya, A., Inukai, N., Narita, T., Wada, T., Sugimoto, S., Konarska, M. M., and Handa, H. (2001) Science 293, 124–127
10. Benzer, D. B., Yamaguchi, Y., Wada, T., Handa, H., and Price, D. H. (2001) J. Biol. Chem. 276, 42601–42609
11. Tan, S., Aso, T., Conaway, R. C., and Conaway, J. W. (1994) J. Biol. Chem. 269, 25684–25691
12. Leib, L., Ren, D., and Burton, Z. F. (1999) Mol. Cell. Biol. 19, 8372–8382
13. Bengel, E., Flores, O., Krauskopf, A., Reinberg, D., and Aloni, Y. (1991) Mol. Cell. Biol. 11, 1185–1206
14. Iizasa, M. G., and Luse, D. S. (1991) J. Biol. Chem. 267, 13647–13655
15. Price, D. H., Sluder, A. E., and Greenleaf, A. L. (1989) Mol. Cell. Biol. 9, 1465–1475
16. Funk, J. D., Nedalkov, Y. A., Xu, D., and Burton, Z. F. (2002) J. Biol. Chem. 277, 46998–47003
17. Yan, Q., Moreland, R. J., Conaway, J. W., and Conaway, R. C. (1999) J. Biol. Chem. 274, 35668–35675
18. Dvir, A. (2002) Biochim. Biophys. Acta 1577, 208–223
19. Shapiro, D. J., Sharp, P. A., Wahl, W. W., and Keller, M. J. (1988) DNA (New York) 7, 47–55
20. Wang, B. Q., Kostruh, C. F., Finkelestein, A., and Burton, Z. F. (1993) Protein Expression Purif. 4, 297–214
21. Wang, B. Q., Lei, L., and Burton, Z. F. (1994) Protein Expression Purif. 5, 476–485
22. Johnson, K. A. (1993) Annu. Rev. Biochem. 62, 685–713
23. Nedalkov, Y. A., Gong, X. Q., Yamaguchi, Y., Handa, H., and Burton, Z. F. (2003) Methods Enzymol., in press
24. Park, J. E., Holmes, S. F., and Erie, D. A. (2001) Cell 106, 243–252
25. Dunlap, C. A., and Tsai, M. D. (2002) Biochemistry 41, 11226–11235
26. Kuzmice, P. (1996) Anal. Biochem. 237, 260–273
27. Patel, S. S., Wong, L., and Johnson, K. A. (1991) Biochemistry 30, 511–525
28. Wong, I., Patel, S. S., and Johnson, K. A. (1991) Biochemistry 30, 526–537
29. Shinwari, A. K., and Tsai, M. D. (2002) Biochemistry 41, 10571–10576
30. Guthold, M., and Erie, D. A. (2001) Chembiochem 2, 167–170
31. Guajardo, R., Lopez, P., Dreyfus, M., and Sousa, R. (1998) J. Mol. Biol. 281, 777–792
32. Guajardo, R., and Sousa, R. (1997) J. Mol. Biol. 265, 8–19
33. Ferrie, N. R., Izak, D., Woodcock, G. R., Wuite, G. J., and Bastamante, C. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 11682–11687
34. Wang, M. D., Schmitz, M. J., Yin, H., Landick, R., Gelies, J., and Block, S. M. (1998) Science 282, 902–907
35. Wang, H. Y., Elston, T., Mogilner, A., and Oster, G. (1998) Biophys. J. 74, 1186–1202
36. Yin, H., Wang, M. D., Svoboda, K., Landick, R., Block, S. M., and Gelies, J. (1995) Science 270, 1653–1657
37. von Hippel, P. H. (1996) Science 281, 660–665
38. Julicher, F., and Bruinsma, R. (1998) Biophys. J. 74, 1169–1185
39. Adelman, K., La Porta, A., Santangelo, T. J., Lis, J. T., Roberts, J. W., and Wang, M. D. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 15358–15354
40. Li, Y., Kordover, S., and Waksman, G. (1998) EMBO J. 17, 7514–7525
41. Li, Y., and Waksman, G. (2001) Protein Sci. 10, 1225–1233
42. Doudleb, S., Tabor, S., Long, A. M., Richardson, C. C., and Ellenberger, T. (1998) Nature 391, 251–258
43. Arndt, J. W., Gong, W., Zheng, X., Shiwalter, A. K., Liu, J., Dunlap, C. A., Lin, Z., Paxson, C., Tsai, M. D., and Chan, M. K. (2001) Biochemistry 40, 5368–5375
44. Fu, T. B., and Taylor, J. (1993) J. Virol. 67, 6965–6972
45. Chang, J., and Taylor, J. (2002) EMBO J. 21, 157–164
46. Macnaughton, T. B., Shi, S. T., Modahl, L. E., and Lai, M. M. (2002) J. Virol. 76, 3920–3927
47. Modahl, L. E., Macnaughton, T. B., Zhu, N., Johnson, D. L., and Lai, M. M. (2000) Mol. Cell. Biol. 20, 6030–6039
48. Zhang, J., Campbell, E. A., Minakhin, L., Richter, C., Severinov, K., and Darst, S. A. (1999) Cell 98, 811–824
49. Vassylyev, D. G., Sekine, S., Borukhov, S., and Yokoyama, S. (2002) Nature 417, 712–719
50. Cramer, P., Bushnell, D. A., and Kornberg, R. D. (2001) Science 292, 1863–1876
51. Epshtein, V., Mustaev, A., Markovtsev, V., Bereshchenko, O., Nikiforov, V., and Goldfarb, A. (1998) Cold Spring Harbor Symp. Quant. Biol. 63, 337–345
52. Huang, J., and Sousa, R. (2000) J. Mol. Biol. 303, 347–358