The nucleosome is generally found to be a strong barrier to transcript elongation by RNA polymerase II (pol II) in vitro. The elongation factors TFIIF and TFIIS have been shown to cooperate in maintaining pol II in the catalytically competent state on pure DNA templates. We now show that although TFIIF or TFIIS alone is modestly stimulatory for nucleosome traversal, both factors together increase transcription through nucleosomes in a synergistic manner. We also studied the effect of TFIIF and TFIIS on transcription of nucleosomes containing a Sin mutant histone. The Sin point mutations reduce critical histone-DNA contacts near the center of the nucleosome. Significantly, we found that nucleosomes with a Sin mutant histone are traversed to the same extent and at nearly the same rate as equivalent pure DNA templates if both TFIIS and TFIIF are present. Thus, the nucleosome is not necessarily an insurmountable barrier to transcript elongation by pol II. If unfolding of template DNA from the nucleosome surface is facilitated and the tendency of pol II to retreat from barriers is countered, transcription of nucleosomal templates can be rapid and efficient.

It is now appreciated that control of transcript elongation is an important aspect of gene regulation (recently reviewed in Ref. 1). A major checkpoint occurs as RNA polymerase II (pol II)2 passes from initiation into productive transcript elongation, ~50 bp downstream of the transcription start site in many genes (2–6). This checkpoint is roughly coincident with the initial contact between pol II and the first nucleosome of the transcription unit (7–9). Once pol II crosses this initial checkpoint, it can elongate transcripts efficiently over hundreds of kilobases of predominantly nucleosomal template (10). The rate of transcript elongation in vivo (10) slightly exceeds the maximum rates reported for transcript elongation by pol II on pure DNA templates in vitro (11, 12). To understand both the initial elongation checkpoint and the exceptional efficiency of transcript elongation downstream of that point, it is essential to develop in vitro systems that duplicate these processes. Progress toward this goal has been somewhat limited because even single nucleosomes have generally proven to be strong barriers to transcribing pol II in vitro (11, 13–17).

Given the tight association of all 146 bp of nucleosomal DNA with the histone octamer surface, one might initially imagine that the nucleosome presents an essentially continuous barrier to transcript elongation by pol II. However, studies with templates bearing single, precisely positioned nucleosomes indicated that the transcriptional barrier for pol II is more discrete. The strongest pauses for human pol II typically occur 45–55 bp within the nucleosome. After pol II crosses this barrier and the nucleosome dyad, transcription continues largely uninhibited through the remainder of the nucleosome (13). This suggests that traversal is controlled primarily by the unfolding of the template DNA from the octamer surface as pol II enters the central segment of the nucleosome organized by the histone H3/H4 tetramer (see Ref. 18).

This idea is supported by the results of recent studies on the transcription of nucleosomes that were altered to facilitate template unfolding. The two strongest protein-DNA contacts in the nucleosome immediately flank the nucleosome dyad (19). These contacts are specifically disrupted by point mutations in histone H3 or H4 called Sin mutants (20). We have recently shown that nucleosomes containing Sin mutant histones do provide a significantly lower barrier to nucleosome traversal by pol II (21). Single-nucleosome unfolding studies have demonstrated that removal of the N-terminal tail domains from histones H2A and H2B specifically favors DNA unwrapping 45–55 bp within the nucleosome (22). We have also shown that overall nucleosome traversal by human pol II is increased and that the +45 barrier in particular is decreased with nucleosomes lacking the H2A/H2B tails (23). These alterations in nucleosome structure are not exact analogs of the events in the nuclei of higher eukaryotes that control transcription elongation. However, results with nucleosomes containing Sin mutant or tailless histones do demonstrate directly that targeted unfolding of DNA from the octamer surface has the potential to play a major role in effective transcript elongation in vivo.

Although facilitating unfolding of critical histone-DNA contacts clearly improves transcription through nucleosomes by pol II, traversal in the in vitro studies just cited remained incomplete and slow relative to transcription of pure DNA templates (21, 23). It is known that pol II backtracks along the
Effective Nucleosome Traversal Requires Elongation Factors

template when it encounters transcriptional barriers, including arrest sites on pure DNA templates (24, 25) and nucleosomes (17). Thus, effective nucleosome traversal is likely to require factors that hold pol II in the transcriptionally competent configuration. TFIIS, which drives transcript cleavage in backtracked complexes to realign the active site and the 3′-end of the RNA (26, 27), has been shown in a number of studies to facilitate nucleosome traversal by pol II in vitro (11, 13, 17, 21, 23, 28, 29). However, even at saturating levels of TFIIS and long incubation times, traversal of single nucleosomes remains incomplete (23). A potential solution is suggested by the work of Burton and co-workers (30), who showed in single-bond formation assays on pure DNA templates that the initiation and elongation factor TFIIF acts synergistically with TFIIS to maintain pol II in the catalytically competent state. This finding predicts that the combined action of these factors should provide a significant increase in the ability of pol II to transcribe nucleosomal templates. Both TFIIS (28) and TFIIF (31, 32) have been shown to associate with pol II across transcribed regions in mammalian cells, so it is reasonable to suppose that they participate in facilitating nucleosome traversal in the cell.

Here, we show that although TFIIS and TFIIF are stimulatory on their own in facilitating nucleosome traversal, these factors clearly cooperate in supporting transcription through nucleosomes by pol II. Importantly, when template unfolding is facilitated by the presence of a Sin mutant histone, the combination of TFIIS and TFIIF allows pol II to traverse nucleosomes efficiently and at rates approaching those seen on pure DNA templates.

EXPERIMENTAL PROCEDURES

Proteins and Factors—The human general transcription factors TATA-binding protein, TFIIF, and TFIIE were recombinant proteins prepared as described (33, 34). Recombinant human TFIIF subunits were expressed, purified, and assembled as described by Wang et al. (35, 36) with the addition of a size-exclusion chromatography step at the end of the purification. TFIIH was purified from HeLa nuclear extract based on the procedure of Maldonado et al. (37) with the following modifications. Nuclear extract was loaded onto a phosphocel- lulose column at 0.1 M KCl, washed extensively at 0.3 M KCl, and developed with a linear gradient of 0.3–1 M KCl in 20 mM Tris (pH 7.9), 1 mM dithiothreitol, 0.2 mM EDTA, 0.25 mM phenylmethylsulfonyl fluoride, and 20% glycerol. TFIIH activity eluted at ~0.5 M KCl. The TFIIH-containing fractions were further purified by step elution from a DE52 column at 0.35 M KCl. The DE52 eluate was then loaded onto a Mono Q column at 0.1 M KCl, which was developed with a linear gradient of 0.1–0.8 M KCl in the same buffer used for the phosphocellulose column. TFIIH activity eluted from Mono Q between 0.25 and 0.35 M KCl. Human pol II was purified from HeLa cell nuclear pellets by the protocol of Maldonado et al. (37) with the modifications described by Újjvári and Luse (33). Human TFIIS was a recombinant GST fusion protein purified by binding and elution from glutathione-agarose. All of the histones used were recombinant Xenopus proteins prepared as described (38, 39). Histones lacking N-terminal tails contained amino acids 13–118 (H2A) or 24–122 (H2B). The Sin mutant H3 has a substitution of isoleucine for threonine at position 118.

DNA Templates, Nucleosome Assembly, and Transcription Complex Assembly—DNA templates were composed of the adenovirus major late promoter upstream and either the 603 or 603R assembly element downstream (13), with an overall length of 255 bp. Working templates were prepared by PCR amplification with the upstream primer biotinylated. Templates were reconstituted into nucleosomes by decreasing salt dialysis. Nucleosome assembly was verified by native gel electrophoresis; typically, only 1–3% of free DNA was detected. For the experiments in Fig. 5, templates were deliberately under-reconstituted, leaving ~25% free DNA. Nucleosomal templates were bound to streptavidin beads and assembled into preinitiation complexes by incubation with human pol II and the general transcript initiation factors as described (13).

Transcription of Nucleosomal Templates—Transcription was initiated with 250 μM CTP, 100 μM UTP, 0.7 μM [α-32P]CTP, and 50 μM dATP at 30 °C for 1 min to generate primarily complexes with 21-mer RNAs (EC21). These complexes were then washed successively with 100 mM KCl and 40 mM KCl. For all reactions except those in supplemental Fig. S4, EC21 complexes were chased for 15–300 s with all four NTPs (1 mM) at 30 °C as indicated in the figures; reactions were terminated by pipetting into phenol-CHCl3. The reactions in supplemental Fig. S4 used 100 or 250 μM NTPs as indicated; all other parameters were the same as in the other figures. Elongation factors (TFIIS, 24 μg/ml; and/or TFIIF, 2 μg/ml) were added along with the NTPs as indicated in the figures. Sarkosyl was added where indicated to a final concentration of 1% prior to the addition of the chase NTPs. The concentration of KCl in all the chase reactions was adjusted to 58 mM to compensate for KCl added with the elongation factors.

Assay for Nucleosome Loss upon Transcription—For the experiment in Fig. 5, templates were radiolabeled at the downstream end. EC21 complexes were synthesized with non-radiolabeled NTPs only and were immediately digested with Dral at 30 °C for 5 min. Reactions for each lane contained 10 ng of template plus 8 units of Dral at a final concentration of 0.8 units/μl. After exchanging the buffer to 100 mM KCl, digestion was continued at 30 °C with 20 units of StyI at a final concentration of 0.8 units/μl for 5 min. Complexes were washed twice with 100 mM KCl buffer containing 0.1% Nonidet P-40 and once with 40 mM KCl buffer. Supernatants from the two digestions and all subsequent washes were saved and combined. Buffer or ρ-amanitin (final concentration of 2.5 μg/ml) was added, followed by TFIIF, TFIIS, and 1 mM NTPs at a final salt concentration of 58 mM KCl as in the other figures. Incubation was continued at 30 °C for 5 min. For the indicated samples, 6.25 units of ClaI was added to a final concentration of 0.2 units/μl; all samples were then incubated at 30 °C for a further 5 min. All reactions were terminated by the addition of phenol-CHCl3. In all cases, labeled RNAs or DNAs were resolved by electrophoresis on denaturing gels and quantified using a STORM Imager and ImageQuant software.
RESULTS

We analyzed the ability of human pol II to transcribe DNA wrapped on the surface of a single, precisely positioned nucleosome. The 255 bp templates consisted of 106 bp promoter segments upstream of 149 bp nucleosome positioning elements. Nucleosomes were reconstituted by salt dialysis, and the nucleosomal templates were attached to beads at the upstream end. Preinitiation complexes were then assembled using purified human pol II and purified or recombinant transcript initiation factors. Transcripts were pulse-labeled to generate complexes with predominantly 21-nucleotide RNAs (EC21). Following rinsing to remove unincorporated label, transcription complexes were chased into the nucleosome with excess non-labeled NTPs. In this study, we focused on the 603 positioning sequence, which directs nucleosome assembly with base pair accuracy (40). We showed previously that nucleosomes reconstituted on the 603 sequence provide a much stronger barrier to transcription when read in one of the two possible orientations. We designated the less permissive orientation 603R and the more permissive orientation 603 (13).

As expected from previous work (13, 23), 603R nucleosomes assembled with wild-type histones provided a very strong barrier to pol II. After a 5-min chase, only ~10% of polymerases reached the end of the template (Fig. 1, A, lanes 1–7). Most of the 603R barrier was concentrated at a closely spaced series of pause sites 45–55 bp within the nucleosome. This barrier resulted from the nucleosome because the addition of Sarkosyl (which removes the nucleosome from DNA) eliminated the barrier (Fig. 1A, lanes 29–35). We tested whether the elongation factors TFIIS and TFIIF could significantly improve transcript elongation by pol II through the 603R nucleosome. The amount of each factor added was saturating as determined by initial titrations on the 603R nucleosomal template. Each factor individually increased traversal by ~2-fold (Fig. 1A and B). When we plotted the time required for the maximum level of run-off transcript to appear for each reaction, it was clear that the rate at which run-off transcript accumulated increased with TFIIF but not with TFIIS (Fig. 1C). This is consistent with the known effects of these factors reported previously for transcript elongation on pure DNA templates (11). In supplemental Fig. S1, we repro-
duced this finding with the template and factor preparations used in this study.

Significantly, the combination of TFIIF and TFIIS greatly improved traversal as compared with the addition of either factor alone (Fig. 1A, lanes 22–28). The amount of run-off roughly doubled (Fig. 1B), whereas the rate at which run-off accumulated was the same as seen with TFIIF alone (Fig. 1C). This agreed with our initial hypothesis that TFIIF and TFIIS should cooperate in facilitating transcript elongation on nucleosomal templates. To confirm that the effect of the elongation factors was not somehow confined to the relatively non-permissive 603R template, we repeated the Fig. 1 experiment with nucleosomes assembled on the 603 sequence. In the absence of factors, traversal was considerably higher on the 603 template as compared with 603R (supplemental Fig. S2, A, lanes 1–7, and B). As we observed with 603R nucleosomes, both TFIIF and TFIIS increased traversal on the 603 template. The two factors combined to stimulate both the level and the rate of traversal. Although TFIIS alone did not eliminate the strong +45 barrier on the 603R nucleosome, on the less restrictive 603 template, TFIIS strongly diminished the central pauses within the nucleosome, particularly in cooperation with TFIIF (supplemental Fig. S2A, compare lanes 7, 14, and 28).

A single amino acid variant of histone H3, the Sin mutant T118I, relieves the requirement of chromatin remodelers for expression of certain genes in yeast (41). Nucleosomes bearing Sin mutant versions of H3 or H4 are less stable and more mobile along the DNA in thermal shift experiments (20). In agreement with our recent findings (21), substitution of H3 T118I for wild-type H3 considerably reduced the traversal barrier imposed by the 603R nucleosome (Figs. 1 and 2A, compare lanes 1–7). Both TFIIF and TFIIS increased traversal of the Sin mutant nucleosome. The overall effects of the factors were generally similar to those shown in Fig. 1 and supplemental Fig. S2. On the H3 T118I-containing template, traversal levels were maximal with TFIIS alone, although the rate of traversal in the presence of TFIIS was stimulated by TFIIF. Note in particular that with both factors, the major +45 barrier was almost completely eliminated (Fig. 2A, compare lanes 1, 7, 14, and 28). A similar overall result was seen with an H3 T118I-containing nucleosome assembled on the 603 template (Fig. 3). The results in Fig. 3 provide an especially clear picture of the differing roles of the two elongation factors. TFIIF

FIGURE 2. Traversal of a 603R nucleosome containing an H3 Sin mutant histone is efficient in the presence of TFIIF and TFIIS. A, same as in Fig. 1, except that the H3 T118I Sin mutant was used for nucleosome assembly instead of wild-type H3. B and C, same as in Fig. 1, except that four repeats were done. Error bars indicate S.D.
increased the rate at which run-off appeared, but all major pause sites were still occupied, even at the longest time points (Fig. 3A, lanes 15–21). TFIIS strongly reduced all of the pauses, but the most effective pause reduction and relatively rapid read-through required TFIIF in addition to TFIIS (Fig. 3A, lanes 8–14 and 22–28).

Single-nucleosome studies demonstrated that removal of the N-terminal tails from histones H2A and H2B facilitates unwrapping of DNA from the histone octamer near the major transcriptional barrier at +45 (22). Our earlier work (21) showed that incorporation of tailless H2A and H2B along with a Sin mutant histone provides an additive increase in nucleosome traversal by human pol II. As shown in supplemental Fig. S3, a histone octamer assembled on the 603R template with tailless H2A/H2B and H3 T118I allowed considerable pol II traversal even in the absence of factors. The addition of either TFIIF or TFIIS stimulated traversal, but the result with both factors together was especially striking: the nucleosomal barrier was essentially eliminated in that case (supplemental Fig. S3, lanes 22–28).

The results in Figs. 2 and 3 and supplemental Fig. S3 suggest an additional question: if the nucleosomal pause sites are nearly eliminated on these templates in the presence of both TFIIF and TFIIS, is transcript elongation through the nucleosome under these conditions equivalent to that seen on free DNA? Fig. 4 shows the results of transcript elongation in the presence of TFIIF and TFIIS on 603R DNA and a 603R nucleosome containing H3 T118I. The efficiency of run-off production was essentially equivalent for nucleosomal and pure DNA templates in this experiment, but even in the presence of saturating elongation factors, some slight pausing was still seen on the nucleosome at the major pause sites near +45. Thus, the traversal rate on the nucleosome was on average about half that on free DNA under these conditions. This represents, to our knowledge, the first demonstration that pol II can achieve full traversal of nucleosomes in a defined biochemical system at rates comparable to those obtained on pure DNA templates. It is important to note that nucleosomes assembled on the 603R sequence are the least permissive for traversal of any we have tested (13).

Because incorporation of the Sin mutant H3 weakens the nucleosome somewhat (20), the results in Fig. 4 might raise a concern that the effective nucleosome traversal simply resulted from the release of the nucleosome from the DNA.

**FIGURE 3.** Traversal of a 603 nucleosome containing an H3 Sin mutant histone is efficient in the presence of TFIIF and TFIIS. A, same as in Fig. 2, except that the 603 nucleosome assembly sequence was used instead of 603R. On this template, there is a major pause 15 bp within the nucleosome (+15), which is indicated to left of the nucleosome (oval) in A. B and C, same as in Fig. 1, except that three repeats were done. Error bars indicate S.D.
upon entry by human pol II. In our earlier work (21), we showed that incorporation of H3 T118I increased the likelihood of nucleosomes dissociating from the template upon traversal by yeast pol II, although only a small fraction of the H3 T118I templates lost nucleosomes at the KCl concentrations used for this study. The procedure that we employed to test for nucleosome loss upon transcription with human pol II and 603R nucleosomes is diagrammed in Fig. 5A. In this experiment, as in our other assays, nucleosome reconstitution was followed by transcription complex assembly and generation of EC21 complexes. In addition to using DNA labeled at the downstream end and non-labeled NTPs, a number of other aspects of the procedure were changed from those employed in Figs. 1–4. Nucleosome release should reveal a unique ClaI restriction site that would otherwise be unavailable for cleavage. To use template cutting by ClaI as evidence for nucleosome loss, it was necessary to overcome two limitations of our system. First, in vitro transcription by human pol II is inefficient, with only a small fraction of the templates transcribed. (This difficulty prevented the use of the native gel assay that was appropriate in the earlier study (21) with yeast pol II.) To be able to detect nucleosome loss on a small minority of templates, we eliminated non-transcribed DNA by precleavage of the EC21 complexes with DraI; the DraI site is protected only on templates with a stalled polymerase. The second problem involved the extent of nucleosome assembly on our templates. Normally, we wish to have nucleosomes on all templates, and thus, we routinely add a slight excess of histones to DNA in our reconstitution reactions, resulting in the presence of some templates bearing two nucleosomes. Dinucleosomal templates do not compromise our transcription studies because the promoter is occluded, and transcription cannot occur. However, such templates would survive both

dral and ClaI cleavage, thereby incorrectly appearing as transcribed templates with surviving nucleosomes. To eliminate this problem, we performed the reconstitutions for the Fig. 5 study in DNA excess, resulting in ~25% free DNA. These templates were then eliminated from subsequent analysis by cleavage at a unique Styl site. As summarized in the schematic at the top of Fig. 5A, treatment of the EC21 complexes with both DraI and Styl leaves only transcription complexes with a nucleosome downstream. These complexes were then chased with excess NTPs in the presence of saturating TFIIF and TFIIS, followed by cleavage with ClaI to detect the loss of nucleosomes. To link nucleosome loss to transcription, the chase reactions were performed with and without amanitin; to determine whether nucleosome loss was greater with the Sin mutant nucleosomes, we compared results with H3 T118I and wild-type H3 nucleosomes. The results of one such experiment are shown in Fig. 5B, and the overall results are presented in Fig. 5C. Some ClaI cleavage was observed in all reactions, but the majority of this apparent nucleosome loss was not transcription-dependent. Importantly, within the error of the experiments, there was essentially no difference in nucleosome loss for wild-type and Sin mutant nucleosomes. Thus, the high levels of traversal of the Sin mutant nucleosomes shown in Fig. 4 did not result simply from nucleosome loss during transcription.

**DISCUSSION**

Many studies have demonstrated that pol II has considerable difficulty in elongating nascent RNAs on nucleosomal templates in vitro (11, 13–17). We show here that TFIIS, which is known to improve traversal through nucleosomes by pol II (11, 13, 17, 21, 23, 28, 29), can be significantly assisted by a complementary elongation factor, TFIIF. We also show
that reducing octamer-DNA interactions through a single amino acid change in one histone can lead to a near abolition of the nucleosomal barrier when the polymerase is assisted by TFIIF and TFIIS. This effect can be magnified by incorporating a second nucleosome alteration, removal of the H2A and H2B tails, which is known to specifically facilitate the unwrapping of DNA from the octamer near the critical barrier (22).

To rationalize these findings, it is useful to consider the molecular basis of the nucleosomal barrier to transcription. As demonstrated in our previous work (13, 21, 23) and in this study, the nucleosomal barrier is focused in the region from about +45 up to the nucleosome dyad. Once pol II has crossed this DNA segment, the downstream template apparently unfolds to allow relatively unimpeded traversal to the end of the nucleosome. (Note that refolding of the upstream DNA onto the octamer surface allows the nucleosome to remain in place as transcription proceeds (18).) In the context of the critical unfolding event, it is important to recall the somewhat surprising tendency of DNA to partially unwrap spontaneously from the octamer surface (42). Of particular interest, this potential window of opportunity for transcript elongation that is created by the unwrapping is expected to last only for 10–50 ms before DNA reassociates with the histones (42).

These last observations suggest that if pol II were poised to take advantage of spontaneous unwrapping, the polymerase should be able to cross the major nucleosomal barrier efficiently. However, other findings would predict that the polymerase will face two major difficulties in taking advantage of the transient unfolding: (i) pol II is likely to backtrack away from the barrier and thus be unable to continue transcription (17); and (ii) particularly in the absence of TFIIF, pol II transcript elongation rates are so low that non-backtracked polymerases will be unlikely to resume elongation before the DNA reassociates with the octamer surface. As shown in supplemental Fig. S1, with saturating levels of TFIIF, about half of the transcription complexes produced a 200-nucleotide run-off RNA in 15 s. We estimate the average elongation rate for polymerases in these reactions at ~600 nucleotides/min, or one bond roughly every 100 ms. Thus, even when transcribing as rapidly as we can currently achieve in vitro, human pol II

**FIGURE 5. Nucleosomes containing Sin mutant histones survive pol II transcription.** A, diagram of the experimental design. Templates containing the 603R sequence were radiolabeled on the downstream end (asterisk) and reconstituted into nucleosomes with wild-type or Sin mutant (H3 T118) histones, deliberately leaving ~25% free DNA. EC21 complexes were formed on ~5–10% of the bead-bound templates. DraI and StyI digestion removed non-transcribed and non-nucleosomal templates, respectively, from the analysis. The washes of the DraI and StyI digestions were collected and show efficient cleavage (see B). Transcription was continued to the end of the template (or inhibited by the addition of α-amanitin (aman.).) Subsequent Clal digestion revealed the amount of free DNA generated during this last incubation step. B, DNA-labeled nucleosomes containing wild-type or Sin mutant (H3 T118I) histones were transcribed by pol II at 58 mM KCl in the presence of TFIIF, TFIIS, and 1 mM NTPs as in Figs. 1–4. The uncut DNA and the cleavage products of the different restriction enzymes are indicated on the right. Note that washing of the beads was able to remove most of the non-transcribed (179-mer, DraI) and non-nucleosomal (84-mer, StyI) DNAs. C, the amount of free DNA generated in reactions as described for B was calculated as the percent Clal digestion product compared with the total amount of DNA in each lane. Error bars indicate S.D. of three trials. Clal digestion of free 603R DNA under the same conditions left only 1–2% undigested DNA (data not shown).
advances at a rate that only approaches the expected rate of reassociation of DNA with the nucleosome surface. It is therefore not surprising that in the absence of TFIIF, pol II is much more likely to backtrack and fall into arrest at critical points in nucleosome traversal. TFIIS allows backtracked polymerases to resume transcription and thereby re-encounter the barrier, but TFIIS does not stimulate overall elongation rates (supplemental Fig. S1) (11). Thus, traversal levels in TFIIS-containing reactions should increase when TFIIF is also present, which is the result that we usually obtained. The importance of rapid transcription in the context of nucleosome traversal is further emphasized by the results of transcription reactions on the 603R-H3 T118I template in the presence of TFIIF and TFIIS with 100 or 250 μM NTPs, instead of the 1 mM NTPs used in all of the other experiments reported here (supplemental Fig. S4). These NTP concentrations are at or below the range reported to support half-maximum elongation rates by mammalian pol II on pure DNA templates (180 – 480 μM NTPs) (43). At these reduced NTP concentrations, at which transcript elongation will be slower, the major +45 barrier was strongly recognized (supplemental Fig. S4). At 100 μM NTPs, essentially all of the polymerases paused at +45, and only a fraction of these were able to continue to run off. Thus, despite the presence of saturating elongation factors and a Sin mutant histone, reducing elongation rates prevents effective nucleosome traversal by pol II.

As shown in Fig. 1A, on the relatively nonpermissive 603R template, a substantial fraction of transcription complexes failed to clear the major +45 barrier in a 5-min reaction even in the presence of both TFIIF and TFIIS. This failure in transcript elongation was nearly eliminated by a single amino acid change in histone H3 (Figs. 1A and 2A, compare lanes 22–28). We have no direct evidence for increased DNA breathing away from the octamer surface in Sin mutant nucleosomes. However, the Sin mutations disrupt critical histone-DNA interactions adjacent to the nucleosome dyad (20), a region that must unfold to allow elongation by pol II complexes paused immediately upstream at the major +45 barrier. Thus, it is reasonable to suggest that transient template unwrapping (and thus, nucleosome traversal) is facilitated by the H3 T118I Sin mutation.

In summary, we have shown that the nucleosome is not necessarily an insurmountable barrier to transcript elongation. Instead, it is a poised gate that can be biased toward opening through relatively modest perturbation. The ability of pol II to cross this gate can also be controlled. In the absence of assisting factors, polymerase is likely to pause and then backtrack within the nucleosome. The addition of factors that drive recovery from backtracking and maintain catalytic competence can allow rapid and efficient crossing of the nucleosomal barrier.

A role for both TFIIF and TFIIS in nucleosome traversal in vivo can be inferred not only from our results and other biochemical evidence (see, in particular, Ref. 12) but also from the co-localization of these factors with pol II in active transcription units within the cell (28, 31, 32). However, other factors that localize within transcribed genes and can facilitate transcript elongation in vitro under some conditions, such as elongin (44, 45) and DSIF (46 – 49), are also likely to assist pol II in maintaining processivity during transcription in vivo. Transient unfolding of DNA away from the octamer surface, which we have stimulated in this study with a Sin mutant histone, could be assisted in vivo by factors. Such factors have not been conclusively identified, but logical candidates would include histone chaperones. Two such factors, FACT and Asf1, are known to associate with the bodies of transcribed genes (50, 51); FACT has been shown to assist nucleosome traversal by pol II in vitro (13, 16, 23). The use of well characterized in vitro transcription systems should provide new insights into the mechanistic roles of these additional factors in efficient transcript elongation by pol II in vivo.

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Effective Nucleosome Traversal Requires Elongation Factors

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