We have previously shown that nucleosomes act as a strong barrier to yeast RNA polymerase II (Pol II) in vitro and that transcription through the nucleosome results in the loss of an H2A/H2B dimer. Here, we demonstrate that *Escherichia coli* RNA polymerase (RNAP), which never encounters chromatin in vivo, behaves similarly to Pol II in all aspects of transcription through the nucleosome in vitro. The nucleosome-specific pausing pattern of RNAP is comparable with that of Pol II. At physiological ionic strength or lower, the nucleosome blocks RNAP progression along the template, but this barrier can be relieved at higher ionic strength. Transcription through the nucleosome by RNAP results in the loss of an H2A/H2B dimer, and the histones that remain in the hexasome retain their original positions on the DNA. The results were similar for elongation complexes that were assembled from components (oligonucleotides and RNAP) and elongation complexes obtained by initiation from the promoter. The data suggest that eukaryotic Pol II and *E. coli* RNAP utilize very similar mechanisms for transcription through the nucleosome. Thus, bacterial RNAP can be used as a suitable model system to study general aspects of chromatin transcription by Pol II. Furthermore, the data argue that the general elongation properties of polymerases may determine the mechanism used for transcription through the nucleosome.

In eukaryotes, the DNA within the nucleus is packaged by histones into nucleosomes. In each nucleosome core, 146 bp of DNA are wrapped in about 1.2 superhelical turns around an octamer containing two molecules each of histones H2A, H2B, H3, and H4. The nucleosome core has a tripartite structure, with the H3/H4 tetramer organizing the central —90 bp of nucleosomial DNA and two H2A/H2B dimers bound to the ends of the nucleosomal DNA (1).

Nucleosomes are present in vivo even when genes are actively transcribed by RNA polymerase II (Pol II), indicating that even if nucleosomal structure is disrupted during transcription, recovery occurs almost immediately after passage of the enzyme (see Refs. 2 and 3 for reviews). Analysis of a Pol II encounter with a mononucleosome resulted in the discovery that the nucleosome acts as a potent barrier to the core Pol II enzyme in vitro, which cannot be relieved even when transcription is conducted in the presence of general elongation factors (4, 5). More recently, it has been shown that transcription through the nucleosome by Pol II results in the loss of an H2A/H2B dimer without changing the position of the histones on the DNA (6).

The bacteriophage SP6 and T7 RNA polymerases have been extensively used as model systems for the in vitro analysis of transcription through the nucleosome (7). The properties of nucleosomal transcription by the multisubunit Pol II and the single-subunit phage SP6 RNAP are dramatically different. The nucleosome is a relatively weak barrier to transcription by bacteriophage RNAP (8–10), and during transcription, the complete histone octamer is transferred from in front of to behind the enzyme (11–15). A nucleosome transfer mechanism is also probably used during DNA replication (16–18) and nucleosome mobilization by ATP-dependent remodeling enzymes (see Ref. 19 for a review). Remarkably, the multisubunit eukaryotic RNA Pol III transcribes nucleosomal templates in a similar manner to SP6 RNAP (15). Thus, different RNAPs use different mechanisms to overcome nucleosomes in vitro (6).

It is surprising that the bacteriophage RNAP is able to transcribe a nucleosomal template more efficiently than Pol II, since all of the enzymes analyzed in vitro, only Pol II is likely to actually encounter nucleosomes in vivo. Indeed, bacteriophage RNAPs never encounter chromatin in vivo; Pol III transcribes only short genes covered with initiation factors that may exclude nucleosomes (reviewed in Ref. 20). The difference between Pol II, on one hand, and bacteriophage RNAPs and Pol III, on the other hand, could be a special evolutionary acquisition developed by Pol II to broaden factor-dependent regulation of genes in vivo. Indeed, this view is supported by the discovery of several transcription factors that facilitate Pol II transcription through chromatin in vitro and in vivo (see Ref. 21 for review). Alternatively, the low efficiency of transcription through nucleosomes by Pol II in vitro as compared with Pol III and phage RNAPs can be due to the differences in the properties of elongation complexes (ECs) formed by these RNAPs. To distinguish between these two possibilities, Pol II was compared with *Escherichia coli* RNAP, which does not transcribe chromatin templates in vivo but otherwise has similar transcription elongation properties to Pol II.

Pol II and *E. coli* RNAP belong to the class of RNAPs that are responsible for synthesis of messenger RNA in prokaryotes and eukaryotes. Although subunit composition varies in these enzymes, they all have similar protein architecture and a com-
mon catalytic mechanism (22). The amino acid composition of the two largest catalytic subunits is very similar among all members of the family (23). General elongation properties of E. coli RNAP and Pol II ECs on DNA are very similar (24, 25). At the same time, E. coli RNAP is a prokaryotic enzyme that never encounters nucleosomes in vivo. Here we test whether the enzyme from E. coli resembles SP6 RNAP and Pol II or uses the different type of mechanism to transcribe through nucleosomes.

To address this question, transcription of nucleosomal templates by E. coli RNAP was analyzed. The data obtained using E. coli ECs clearly indicate that this enzyme utilizes the Pol II-type mechanism. All distinct features of the mechanism, including higher nucleosome barrier to transcription, lack of nucleosome translocation, and displacement of an H2A/2B dimer, were recapitulated with E. coli RNAP. These data suggest that the general elongation properties shared by all members of the Pol II family may determine the mechanism of transcription through the nucleosome and the fate of the histone octamer.

**EXPERIMENTAL PROCEDURES**

**Nucleosome and Hexosome Reconstitution, Purification, and Analysis**—Nucleosomes and hexosomes were assembled on the 204-bp pVT1 TspRI-StaI fragment by octamer exchange as described (6). Hexosomes were gel-purified as described (6). Nucleosome mapping was performed as described (6).

**Transcription Buffers**—TB contains 20 mM Tris-HCl (pH 7.9), 5 mM MgCl₂, and 1 mM β-mercaptoethanol. The numerical index to the KCl concentration (mM) refers to the KCl concentration (mM).

**EC Assembly and Ligation to the 204-bp DNA or Nucleosomal Template**—Hexadhistidine-tagged Pol II and E. coli RNAP were purified according to published protocols (25, 26). The assembly and ligation were done as described (6, 25, 27), with the following modifications: 3 pmol of core RNA/DNA Pol II was incubated with 13–26 pmol of duplex (the 5' end primer RNA) (RNA9) annealed to the 50-nt template DNA strand (TDS50)), and the fully complementary nontemplate DNA strand (nontemplate DNA strand 59 (1.3–2.6 nmol) was incorporated as described (25, 27). The Ni²⁺/NTA-agarose (50 ml of 50% suspension; Qiagen, Chatsworth, CA) was washed three times with TB40, pre-treated with 0.5 mg/ml acetylated BSA (Sigma) for 10 min, and washed again two times with 1 ml of TB40. EC9 was immobilized on the resin by constant shaking for 15 min at room temperature, washed three times with 1 ml of TB40, incubated for 10 min in 1 ml of TB1000, and washed twice with 1 ml TB40. For analyzing the templates after transcription, EC45 was formed as described (6, 8). Mobility controls with the hexasome or nucleosome ligated to the 50-nt DNA used for assembly or the 204-bp DNA duplex used for assembly and the 110-bp T7A1 fragment were prepared as described (6). Quantitation was performed as described above.

**RESULTS**

**Assembly of E. coli RNAP ECs and Ligation to Defined Nucleosomal Templates**—To determine the mechanism of transcription through the nucleosome used by E. coli RNAP and to compare it with the Pol II-type mechanism, transcription was performed with both enzymes under very similar conditions using promoter-initiated and assembled ECs (6) (Fig. 1A). Briefly, ECs were assembled by annealing a 50-nt template DNA strand (TDS50) to a 9-nt RNA oligonucleotide (RNA9) and allowing the core polymerase to bind (27). The transcription bubble was formed by incorporation of the 59-nt nontemplate DNA strand into the complex. This created a long 3’-overhang (TspRI site) to be used for ligation. Finally, the completed EC9 (with the numerical index referring to the length of the RNA in the EC) was immobilized on Ni²⁺/NTA-agarose beads through the hexadhistidine tag at the carboxyl terminus of the β' subunit of RNAP (26) or at the amino terminus of the Rpb3 subunit of Pol II (24). Alternatively, promoter-initiated EC11 was formed by incubation of RNAP and 110-bp T7A1 promoter-containing DNA fragment in the presence of ApUpC, ATP, and GTP. EC9 and EC11 were ligated to the 204-bp TspRI-cut DNA or mononucleosomes with defined positions (see Fig. 1, A and B) (6). The polymerase was walked to a point beyond the ligation.
junction (+45), and the ECs were washed. Thus, polymerase from any ECs that were not ligated would run off the template, the unligated templates would be washed away, and only ligated templates would remain in the reaction. Transcription was resumed by the addition of all four NTPs.

The 204-bp template is only large enough to accommodate a single nucleosome per DNA molecule. Despite the fact that this template contained a nucleosome positioning sequence from the Xenopus 5 S RNA gene (6, 28), reconstitution resulted in a mixture of mononucleosomes with different positions along the DNA (Fig. 1B). Nucleosome positioning was previously determined by restriction enzyme mapping and micrococcal nuclease mapping (6). Two regions of the template were preferred locations for nucleosome formation (N1 and N2; N2 is positioned on the 5 S sequence). Each of these regions had two local heterogeneous positions (a and b), giving rise to four different nucleosome positions (N1a, N1b, N2a, and N2b). The N1 and N2 nucleosomes are present in an ~1:1 ratio. The amounts of N1a and N1b are about equal, whereas N2b is present at about 2.5 times the amount of N2a.

The Nucleosome-specific Pausing Patterns Are Similar for E. coli RNAP and Pol II at Physiological Ionic Strength—To estimate the level of similarity in transcription through the nucleosome by E. coli RNAP and Pol II, elongation was performed with both enzymes under very similar conditions. ECs were assembled with 5'-labeled 9-nt RNA oligonucleotide and ligated to the 204-bp nucleosomal template. The resulting template was shown in Fig. 1B. RNAP was walked to the +45-position or in some cases the +48 position using [α-32P]NTPs to label the RNA. Alternatively, the RNA was labeled at the 5'-end prior to EC assembly. The ECs were washed after each manipulation, and transcription was resumed by the addition of all four NTPs. B, nucleosome positioning on the ligated 254-bp template used for assembly (EC9, top) and the 314-bp template used for promoter initiation (EC11, bottom). The 110-bp TspRI-cut fragment of DNA containing the T7A1 promoter was the exact same sequence of DNA used for EC assembly (~10 to +244), except that there was an extra 60 bp of DNA upstream of the promoter (~70 to ~11). The numerical labeling on the template represents the positions along the DNA relative to the transcription start site (+1). Locations of the TspRI, MspI, EcoRI, and EcoRV restriction sites are indicated. The 254-bp template was labeled at the ligation site (TspRI site; star), and the 314-bp template was labeled at the promoter-proximal end (star). The nucleosome positions, N1a, N1b, N2a, and N2b are shown as ovals (a is solid, b is dashed) below the template.
Formation of EC48 (by adding cold UTP and [α-32P]GTP; see the sequence in Fig. 3A), and the ECs were washed again. Transcription was resumed from EC48 with the addition of all four NTPs at different concentrations of KCl. Transcription of histone-free DNA was very efficient and was completed within 5 min at all of the salt concentrations tested (Fig. 3A, lanes 1–6). However, at 150 mM KCl and below, the nucleosome arrested most of the RNAP in the region from +48 (near the N1 nucleosomal border) to +150 (lanes 8 and 9). The minor amount of run-off transcript that was formed under these conditions (<10% of the total radioactivity of the lane) most likely resulted from transcription of the small amount of free DNA present in the nucleosome preparation (<15%). In 300 mM KCl, 25–30% of the RNAP could pass through the nucleosome (lane 10). This moderate ionic strength is enough to weaken some of the histone-DNA interactions, but the nucleosomal structure, EC integrity, and fate of the nucleosome during transcription are still maintained (6, 29, 30). When the ionic strength is raised to 1 M KCl, the nucleosome is destroyed (6, 30), but the EC remains stable (27). Under these conditions, RNAP was able to complete transcription of the template with the same efficiency as that on the naked DNA (compare lanes 5 and 11). Moreover, when transcription was initially conducted at 40 mM KCl, and then the salt concentration was raised to 1 M, more than half of the RNAP that was originally stopped in the nucleosome was able to complete transcription at the higher salt concentration (lane 12). Thus, for the majority of RNAP that could not transcribe through the nucleosome, the ECs were intact, and the arrest was reversible. These properties of nucleosomal transcription, including the pattern of pausing and the strength of the barrier, were strikingly similar to what was observed for Pol II under very similar conditions (6).

Timed courses of transcription on the free DNA template and through the nucleosome were conducted at 300 mM KCl so that a substantial fraction of the RNAP (25–30%) could complete transcription of the nucleosomal template (Fig. 3B). Transcription of the free DNA template was nearly complete after 30 s (lane 3). In contrast, the same time point for the nucleosomal template only had a small amount of run-off transcript produced (probably from transcription of the free DNA contaminating the nucleosome preparation; lane 9). The majority of the RNAP was arrested in the nucleosome. Even at a salt concentration 2 times higher than physiological ionic strength, the rate of transcription through the nucleosome was ~10 times slower than that of the DNA template. Again, this result was remarkably similar to what was seen for transcription through the same nucleosomes by Pol II (6). Moreover, some of the pauses and arrests coincide with the intrinsic pause sites on the naked DNA as they do with Pol II (6, 31).

Nucleosomes Form Similar Barriers to Assembled and Promoter-initiated E. coli ECs—The authenticity of assembled ECs of E. coli and yeast polymerases has previously been verified (24, 25, 27). Assembled ECs of E. coli RNAP were found to be indistinguishable from promoter-initiated ECs in terms of EC stability, footprint size, and rate of RNA polymerization (27). However, it had not been determined whether the mechanisms of transcription through the nucleosome were similar for promoter-initiated and assembled ECs. In our previous work with Pol II (6), we were unable to address this issue because promoter-dependent Pol II in vitro systems are extremely inefficient in terms of initiation (32). We solved this problem using E. coli RNAP, which supports much more efficient promoter initiation than Pol II and requires no additional transcription factors. As illustrated in Fig. 1B, a 110-bp TspRI-cut DNA fragment containing the A1 promoter of bacteriophage T7 was used for this purpose (26). This DNA fragment is identical in sequence to the 50-bp DNA used for EC assembly from the region −10 to the TspRI (ligation) site, but it contains an extra 60 bp of DNA upstream of the template used for EC assembly (−70 to −11; Fig. 1B). Thus, the transcribed sequence is identical for both templates. Promoter initiation was achieved as follows: (i) the 110-bp promoter fragment and E. coli holo-RNAP were incubated at 37 °C to form initiation complexes; (ii) transcription was initiated by adding ApUpC, ATP, and GTP and incubating at 37 °C to stall Pol II before incorporating CTP in the +12-position and allow the formation of EC11; and (iii) the EC was immobilized on Ni2+-NTA-agarose beads and washed. The rest of the experimental procedure is identical to that used for transcription with assembled ECs (Fig. 1A).

The strength and pattern of nucleosome-specific pausing by promoter-initiated E. coli RNAP was compared with that of assembled ECs. This was done by analyzing the efficiency of transcription on the DNA and nucleosomal templates at different KCl concentrations (compare Fig. 4A with Fig. 3A). RNAP was walked from EC11 to EC45, the RNA was labeled during the formation of EC48, and transcription was resumed from EC48 as described for the experiment in Fig. 3A. As for the

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**Fig. 2.** The nucleosome-specific pausing of *E. coli* RNAP is similar to that of Pol II. Time courses of transcription on the nucleosomal template at 150 mM KCl are compared side-by-side for the two enzymes. The RNA was labeled at the 5′-end (marked by an asterisk in the sequence below the gel) prior to EC assembly, and the polymerases were walked to form EC45 and washed. Transcription was resumed at 150 mM KCl by adding all four NTPs for the indicated time periods. The transcribed sequence is shown below the gel. The arrows at the left indicate the 45-nt starting material (lanes 1 and 7), the 64-nt read-through product, and the 244-nt run-off transcript. Nucleosome positions are indicated on the right.
assembled ECs, promoter-initiated RNAP was able to complete transcription of the DNA template at each of the salt concentrations analyzed (lanes 1–6). The nucleosome was again found to be a strong barrier. Full-length transcript formation was prevented at physiological ionic strength or below, and RNAP paused in the region from +48 (near the N1 nucleosomal border) to +150 (lanes 7–9). The strength of the barrier and the nucleosome-specific pausing pattern were indistinguishable between promoter-initiated E. coli RNAP and assembled ECs.

Time courses of transcription on the DNA and nucleosomal templates at 300 mM KCl were also analyzed for promoter-initiated RNAP (Fig. 4B). Again the results were very similar to what was seen for assembled ECs (compare with Fig. 3B). As expected, histone-free DNA transcription was almost complete after 30 s (lane 3), and transcription of the nucleosome was ~10 times slower (lanes 7–12). This similarity was further confirmed by the quantitative analysis of the data (Fig. 4C). Moreover, the same pausing pattern was observed with promoter-initiated ECs as with assembled ECs (compare Figs. 4B and 3B). The majority of the individual pause sites encountered by both E. coli RNAP and Pol II appear to be the same (especially the most prominent pause sites at about +175, 170, 147 (very strong), –140, 125, 113, 105, 92, 88, 80, 75, and 69 nt; see Fig. 2 and Ref. 6). In summary, the mechanisms of transcription through the nucleosomes by promoter-initiated E. coli RNAP, assembled E. coli RNAP ECs, and assembled Pol II ECs are very similar (Figs. 2–4; see also Ref. 6).

Transcription through the Nucleosome by E. coli RNAP
Results in the Loss of an H2A/H2B Dimer—Finally, the fate of the nucleosome during transcription with E. coli RNAP was analyzed using assembled ECs containing labeled DNA (Fig. 5A; see Fig. 1B). EC9 was incubated with ATP, CTP, and GTP, and NTPs to form EC45, and the templates contained in the supernatant were analyzed in a native gel. As was the case for Pol II, a fraction of the ECs dissociated during this walking step, and therefore nontranscribed templates were released into the supernatant (Fig. 5A, lane 1; see Ref. 6). Prior to transcription, the N1 nucleosomes are resistant to cleavage by EcoRI, and N1a is sensitive to cleavage by EcoRV (lanes 2 and 3). N1b is only slightly sensitive to EcoRV, because this restriction enzyme site is right on the nucleosomal border. The N2 nucleosomes are resistant to cleavage by EcoRV and sensitive to cleavage by EcoRI. The nucleosomes do not repose during ligation to the ECs (Fig. 1B) (6). Next, EC45 was
sensitive to EcoRI and resistant to EcoRV, whereas the others were sensitive to EcoRV and resistant to EcoRI (data not shown; see Fig. 1B). Thus, transcription though the nucleosome by E. coli RNAP led to the loss of an H2A/H2B dimer and the formation of a hexasome. This is the same mechanism that was seen for Pol II transcription through the nucleosome (6).

Our assay for determining the fate of the nucleosome after transcription relies, in part, on the mobility of the template during native gel electrophoresis. Mobilities of nucleosomes in native gel depend on their positions on the DNA (33). Note that on the 254-bp template, the N1 and N2 nucleosomes are resolved in a native gel, but the local heterogeneous positions of the nucleosomes are indistinguishable. N1a is not resolved from N1b, and N2a is barely resolved from N2b (Fig. 5A, lane 4). After the addition of all four NTPs, primarily fully transcribed templates were released into the supernatant (Fig. 5A, lane 5). This was also confirmed by labeled transcript analysis, where the primary RNA product released into solution at this step was full-length run-off (not shown; see Ref. 6). Transcription through the nucleosome resulted in the appearance of a faster migrating band (lane 5, *Hex*.) as compared with the mobility of the original nucleosomes (N1 and N2, lane 1). This band had the same mobility in a native gel as the reconstituted hexasome control (lane 6, see “Experimental Procedures”), suggesting that an H2A/H2B dimer was lost during transcription. The addition of histones H2A and H2B back to the transcribed templates led to the restoration of nucleosomes with mobilities similar to N1 and N2, indicating that the surviving histones remained bound at their original positions on the DNA (Fig. 5B, lanes 1 and 2). As expected, some of the transcribed templates were sensitive to EcoRI and resistant to EcoRV, whereas the others were sensitive to EcoRV and resistant to EcoRI (data not shown; see Fig. 1B). Thus, transcription though the nucleosome by E. coli RNAP led to the loss of an H2A/H2B dimer and the formation of a hexasome. This is the same mechanism that was seen for Pol II transcription through the nucleosome (6).

The fate of the nucleosome during transcription by promoter-initiated and assembled E. coli ECs were compared (Fig. 6B). To allow for only a single round of transcription, rifampicin was added to the transcription reaction to block reinitiation (34). EC11 was walked to EC45. Any nontranscribed templates released from ECs that fell apart as a result of transcription of the promoter-proximal region (lane 1) were washed away. Note that the fraction of unstable ECs is much less for promoter initiation than for EC assembly (compare Fig. 6B, lane 1, with Fig. 5A, lane 1). The mock transcription control (Fig. 6B, lane 2) illustrates that any EC dissociation is at a background level after washing. Finally, with the addition of all four NTPs, the
completely transcribed templates are released (lane 3). The mobility of the transcribed nucleosome is faster than the original nucleosomes (lane 4) and comparable with that of the reconstituted hexasome (lane 5). Furthermore, DNA controls do not give rise to a band with hexasome mobility in the native gel (data not shown). Thus, similar mechanisms of transcription through the nucleosome are utilized by promoter-initiated and assembled ECs, each resulting in the formation of hexasome. This is the same mechanism that was seen for Pol II. Moreover, as for transcription with Pol II (6), transcription in solution using EC45 eluted with imidazole gives the same result (data not shown), indicating that the formation of hexasome occurs both during transcription in solution and while using the immobilized system.

DISCUSSION

Here, we report that RNA polymerase from E. coli recapitulates all of the properties of nucleosomal transcription by yeast Pol II in vitro (6). The nucleosome imposes a very strong barrier for bacterial RNAP that is partially reduced at elevated salt concentration. The pausing starts within the first 20 bp of the nucleosomal DNA and spreads almost across its entire length. The nucleosome-induced pausing of E. coli RNAP is very similar to that of Pol II in terms of strengths of the pauses and pausing patterns. Moreover, the passage of E. coli RNAP causes quantitative removal of one H2A/H2B dimer from the histone octamer, without changing the position of the remaining histones on the DNA. These results are highly similar to what was obtained with Pol II under identical conditions on the same nucleosomal template (6).

Our previous data with Pol II relied entirely on the ability to assemble Pol II ECs in the absence of initiation factors and promoter sequence (6). It has not been verified if the elongation properties of the assembled ECs on the nucleosomal template are the same as those of the "native" promoter-initiated ECs. As we show here, the properties of transcription through the nucleosome by promoter-initiated E. coli RNAP and assembled ECs are indistinguishable (compare Figs. 3 and 4 as well as Figs. 5 and 6). This similarity further establishes EC assembly as a valid and credible technique for studying transcription through the nucleosome in vitro.

The similarity of the mechanisms of transcription through the nucleosome by E. coli RNAP and yeast Pol II is most likely explained by the high structural and functional similarity of these enzymes. Both polymerases have very similar structure, and the amino acid composition of the active center is similar across almost the entire length of the template/transcript-binding site (23, 35, 36). In fact, the amino acid composition of these sites is almost 90% identical in the E. coli and yeast enzymes (23, 35).

Reflecting a close similarity in protein design, the nucleic acid architecture is also very similar in bacterial and yeast Pol II ECs. These ECs protect 10–12 nt of double-stranded DNA and contain a 10–12-nt melted segment that forms the transcription bubble (25, 36). In the bubble, 8–9 nt of the 3’-proximal RNA hybridize with the template DNA strand to form an RNA:DNA hybrid, which represents a major stability determinant in both ECs (25, 27, 37). Two DNA strands come together 1–2 nt downstream and 2–3 nt upstream from the
RNA:DNA hybrid. Both polymerases are believed to use the same "ratchet" mechanism for translocation along the DNA (36, 38). In addition to forward movement, both enzymes are capable of backward motion without degrading the nascent RNA. This reaction occurs by backward sliding of the enzyme along the template with extrusion of the 3′-end of the RNA from the active center and results in transcription arrest (24, 39). Notably, it has been demonstrated that neither Pol II nor E. coli RNA polymerase dissociate from the template upon the encounter with the nucleosome, indicating that the DNA-bound histones may induce transcription arrest of these polymerases. It is known that Pol II and E. coli RNA polymerase ECs respond similarly to arrest sites and various roadblocks (40–42).

The high sensitivity of Pol II and E. coli RNA polymerase ECs to various roadblocks, including nucleosomes, could be explained by the arrest caused by backtracking over a large distance (10 nt or more; see Refs. 24, 43, and 44 and references therein). In contrast, an exceptionally high elongation rate of the bacteriophage RNAPs (45), taken together with a very limited (not more than 2–3 nt) lateral movement of the polymerase in the EC (46), suggests that the ECs are likely to be resistant to arrest induced by the roadblocks. Indeed, SP6 RNAP can efficiently overcome the nucleosomal barrier to transcription (6, 8, 29). Similarly, yeast Pol III ECs are also much less sensitive to various roadblocks (47, 48), including nucleosomes (15). Thus, the differences in catalytic properties or propensity to backtrack of the various RNAPs may account for the differences in the efficiency of transcription through the nucleosome by these enzymes. The fact that E. coli RNA polymerase encounters the same block during transcription through the nucleosome as Pol II may be indicative that it is also regulated to some degree by DNA packaging. E. coli RNA polymerase does not encounter nucleosomes in vivo; nonetheless, the bacterial DNA is highly compacted by basic proteins (49, 50) that can possibly form a barrier to transcribing RNA.

E. coli RNA polymerase represents one of the most simply organized members of its family, which makes it particularly valuable for the study of the mechanism of nucleosomal transcription. The catalytic core of the bacterial enzyme, capable of normal elongation and termination, is made of two large β and β′ subunits and a dimer of α subunits, which correspond to RPB2, RPB1, and a heterodimer of two RPB3/RPB5 subunits in the yeast Pol II, respectively (23, 36). There is a vast pool of already known mutants of E. coli RNA polymerase with altered elongation properties, and a well established genetic system makes it easy to obtain and characterize novel mutants (51). Moreover, the biochemical system allows for reconstitution of E. coli RNA polymerase from either separately purified subunits or even separate domains within the subunits, which extends the list of manipulations possible with mutant RNAPs in vitro (52). These advantages of the model system based on E. coli RNA polymerase may promote identification of the subunits or structural domains responsible for specific properties of transcription of nucleosomal templates by bacterial RNA polymerase and Pol II.

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