The elongation rate of RNA polymerase determines the fate of transcribed nucleosomes

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Abstract

Upon transcription, histones can either detach from DNA or transfer behind the polymerase through a process believed to involve template looping. The details governing nucleosomal fate during transcription are not well understood. Our atomic force microscopy images of RNA polymerase II-nucleosome complexes confirm the presence of looped transcriptional intermediates and provide mechanistic insight into the histone-transfer process via the distribution of transcribed nucleosome positions. Significantly, we find that a fraction of the transcribed nucleosomes are remodeled to hexasomes, and that this fraction depends on the transcription elongation rate. A simple model involving the kinetic competition between transcription elongation, histone transfer, and histone-histone dissociation quantitatively rationalizes our observations and unifies results obtained with other polymerases. Factors affecting the relative magnitude of these processes provide the physical basis for nucleosomal fate during transcription and, therefore, for the regulation of gene expression.

DNA in eukaryotic cells is tightly wrapped into nucleosomes, which constitute a physical barrier for RNA polymerase II (Pol II) and function as important and ubiquitous regulators of transcription elongation[1–3]. *In vivo*, nucleosomes are disrupted to varying degrees by transcription elongation, with outcomes ranging from partial loss to complete removal and exchange of histones[4–9]. Since these different outcomes can influence further binding of chromatin remodeling factors and the advancement of subsequent transcribing polymerases on that gene[10], it is important to understand the mechanistic details that determine the fate of the nucleosome during transcription.

In vitro studies with the phage SP6 RNA polymerase and RNA Polymerase III (Pol III) have shown that, upon transcription, the histone octamer moves upstream by 40–95 base pairs[11–13]. Surprisingly, later experiments suggested that transcription through a nucleosome by Pol II leads to H2A–H2B dimer loss, and the formation of a hexamer whose position on DNA is unchanged[14,15]; similar results were obtained with the *E. coli* RNA polymerase[16]. This transfer process is believed to involve looping of the DNA template, but claims of template looping for Pol II have so far relied on indirect evidence[17–19]. Moreover, despite extensive work on characterizing the nucleosomal barrier[11–18,20–25], there is still little mechanistic understanding of how transcription dynamics affects histone turnover, and little basis for rationalizing differences among polymerases.

Here, we describe experiments that make it possible to image concurrently the polymerase and the nucleosome with atomic force microscopy (AFM) in order to obtain snapshots of individual Pol II-nucleosome complexes before, during and after transcription. These images allow us to directly visualize the nucleosome integrity and its position after transcription, to look for DNA looping during histone transfer, and to explore conditions that favor partial versus complete histone transfer.

RESULTS

Identification of transcribed complexes

Briefly, Pol II elongation complexes were assembled on a 96 bp DNA template[14,17] and ligated to 574 bp of DNA containing a single nucleosome loaded on the 601 nucleosome positioning sequence (NPS)[26] (Methods, Supplementary Fig. 1). Complexes prepared in this manner were incubated either in the absence ("stalled sample," Fig. 1a) or presence ("chased sample," Fig. 1b) of nucleotide triphosphates (NTPs), then fixed with formaldehyde, deposited on mica, and imaged using AFM (Methods). Because Pol II has a significantly larger molecular weight (~550 kDa) than the nucleosome (~190 kDa), it is possible to unambiguously distinguish the two complexes by their different sizes in the images (Fig. 1a–c). We measured the lengths of the different segments of free DNA (i.e. the DNA not covered by protein, Fig. 1c), as well as the heights of the proteins for complexes that have both the nucleosome and the polymerase[27] (Methods).

The position of the polymerase in stalled samples is centered at the start site of transcription (Fig. 1a, Supplementary Fig. 2a). In contrast, after addition of all four nucleotides, Pol II is distributed along the entire length of the DNA template, indicating that transcription has ensued (Fig. 1b, Supplementary Fig. 2b).

To determine which complexes in the chased sample have completed transcription through the nucleosome, we made use of the fact that the DNA upstream of the nucleosome, containing the start site of Pol II, is about three times longer than the DNA downstream of the nucleosome. When Pol II is on the long arm and does not contact the nucleosome, we reason that transcription has not yet proceeded into the nucleosomal region, and label these nucleosomes as "untranscribed". Conversely, when Pol II is on the short arm, we infer that transcription through the nucleosome was completed, and we deem these nucleosomes "transcribed" (Fig. 1d, Supplementary Fig. 2b). In order to correctly identify transcribed nucleosomes, we assume that even if their positions change from the original NPS, they remain on the same half of DNA after transcription. We use a boot-strapping method to check this assumption: if the nucleosome moved on the other half of the DNA, we would expect a change in the position distribution of nucleosomes that we deemed untranscribed. However, for complexes identified as untranscribed in the chased sample, the position of the nucleosome is unchanged compared to untranscribed nucleosomes imaged in the absence of Pol II (Fig. 2a, $p = 0.3$, t-test), indicating that our identification of these nucleosomes as untranscribed is valid.

Nucleosome position after transcription

In order to get an accurate measurement of the changes in position of the nucleosomes (Methods, Supplementary Discussion 1, Supplementary Table 1), we compared the length of the free DNA segment upstream of transcribed nucleosomes (Fig. 2b, red) to that of untranscribed nucleosomes from a sample without Pol II (Fig. 2b, blue). The distribution for transcribed nucleosomes is broader and there is a modest but statistically significant shift to shorter lengths (6 nm, $p = 5 \cdot 10^{-8}$, t-test). The partial overlap with the corresponding distribution of untranscribed nucleosomes suggests that the majority of the nucleosomes are placed at the same location after transcription, in accordance with previously published results[14]. However, our single-molecule method, used in conjunction with a DNA sequence that positions the nucleosome uniquely, allows us to observe that a small subpopulation of the transcribed nucleosomes (approximately 20%) move immediately upstream of their original position by 24 nm (72 bp) on average (Fig. 2b). This upstream relocation of the histones hints at a looping mechanism of histone transfer, mechanism initially proposed for phage polymerases, which were used as a substitute system for eukaryotic transcription[11,21], and more recently for Pol II[17].

DNA looping during histone transfer

In models of DNA looping during nucleosomal transcription, the histones from a partially unwrapped nucleosome situated downstream of the transcribing polymerase are assumed to simultaneously contact a DNA segment upstream of the polymerase forming a loop. According to such models, this process eventually leads to the transfer of histones behind the polymerase and permits transcription to resume. In agreement with this idea, we find many intermediate complexes where Pol II is in the process of transcribing the nucleosome that show the histones contacting the DNA segments both upstream and downstream of Pol II (Fig. 3a).

The distribution of total free DNA lengths for intermediate complexes where Pol II is in the process of transcribing the nucleosome is different from that of complexes where Pol II has started transcribing but has not yet reached the nucleosome (p = 0.009, Kolmogorov-Smirnov test, see Supplementary Fig. 2 for populations selected). Because the free-DNA lengths distribution for these intermediate complexes is not well described by a single Gaussian (p = 0.03, Lilliefors test), we fit this distribution with two Gaussians (Fig. 3b). The main peak is identical with the corresponding distribution for complexes where Pol II has started transcribing but has not yet reached the nucleosome (Fig. 3c). The second peak corresponds to an additional population of intermediates where the DNA outside the polymerase-nucleosome complex is shorter by ~30 nm. We interpret this shortening as evidence that the template in the proximity of the nucleosome participates in a loop that facilitates histone transfer behind the polymerase, and that cannot be resolved because of the broadening effect of the AFM tip (inset, Fig. 3b). The estimated size of these DNA loops (~90 bp) is smaller than the persistence length of DNA (~150 bp) and they may be facilitated by the putative 90° bend that Pol II introduces in its DNA template[18,19,28,29].

Pol II transcription produces hexamers and octamers

As Pol II advances onto the nucleosomal template, the DNA is being detached from the core histones, exposing them to the surrounding conditions. Since the octamer consists of a collection of positively charged histones, it is unstable at salt concentrations under 1 M[30,31]. Thus, unless the core histones contact another piece of DNA that could neutralize their charges and stabilize their association, the octamer may dissociate with partial loss of its components. Indeed, loss of an H2A–H2B dimer and the formation of a hexasome upon transcription by Pol II has been reported previously[14,15]. Consistent with these results, we observe a reduction in the apparent physical size of transcribed nucleosomes (Fig. 4a). Their height consists of two populations: one similar to untranscribed nucleosomes (3 ±0.4 nm, Fig. 4b) and the other one corresponding to subnucleosomal particles with lower height (2.1 ±0.3 nm, Fig. 4c).

In order to identify the transcribed particles with decreased height, we reconstituted and imaged histone tetramers on DNA using the same methods as for octamers[32], except with H2A and H2B histones omitted. The height of tetramers, 1.6±0.2 nm, is substantially lower than that of the transcribed particle, 2.1±0.3 nm (Fig. 4d). Moreover, when we destabilized complete octameric nucleosomes by incubating them in 1 M KCl, we obtained 3 nucleosomal species, consistent with octamers, hexamers and tetramers (Fig. 4e). The height of the middle peak, which we identify as a hexamer, matches the height of the subnucleosomal particles resulting from transcription.

Hexamers to octamers ratio depends on the elongation rate

Most significantly, we find that the fraction of smaller subnucleosomal particles observed after transcription depends on the rate of elongation. When transcription was performed at low NTP concentrations (100 μM), only 10±3% of the transcribed nucleosomes were converted to hexasomes (Fig. 5a). Increasing the NTPs concentration to 200 μM NTP augments the percentage of hexasomes to 17±3% (Fig. 5b). At saturating NTPs (1000 μM),

25±5% of the transcribed nucleosomes were converted to hexasomes (Fig. 5c). No changes were observed in the sizes of untranscribed nucleosomes in these samples (Fig. 5d–e).

We attribute these different outcomes of transcription to the kinetic competition between histone dissociation from a partially unwrapped nucleosome and histone transfer to the upstream DNA. Initially, as the nucleosome partially unwraps during Pol II advancement, enough of the histone core is exposed to allow contact with the upstream DNA through a temporary DNA loop, but not so much as to cause H2A–H2B dissociation. During slow transcription (100 μM NTPs) this partially exposed histone intermediate lasts long enough to allow transfer of the intact octamer onto the upstream DNA. However, if the rate of transcription is increased slightly, more of the nucleosome will unwrap and, as enough of the histone core becomes exposed, dimer dissociation starts competing with octamer transfer to the upstream DNA. Under these conditions, representative for transcription at 200 μM and 1000 μM NTPs, both octamers and hexamers can be found as a result of transcription. Finally, when the rates of transcription are even higher, enough DNA is unwrapped from the surface of the histone core that the complete histone detachment from DNA greatly outcompetes the rates of histone transfer and histone-histone dissociation, thus leading to bare DNA formation.

Elongation, looping, and histone dissociation compete

The dependence of the outcome of transcription on the speed of elongation indicates that a kinetic competition exists between the net rate of nucleosome unwrapping during elongation (k_{ue}), octamer transfer (k_t), and dimer dissociation (k_d) during transcription through the nucleosome (Fig. 6a).

In this competition model, the probabilities of observing a hexamer (P_{hex}), an octamer (P_{oct}), or bare DNA (P_{bare}) after transcription can be written as:

$$
\begin{aligned}
P_{hex} &= \left(\frac{k_{ue}}{k_t+k_{ue}}\right)^N \left(\frac{k_d}{k_t+k_d}+\frac{k_t}{k_t+k_d}\cdot\left(\frac{k_{ue}}{k_t+k_{ue}+k_d}\right)^{N_T-N}\right) - \left(\frac{k_{ue}}{k_t+k_{ue}}\right)^{N_T}, \\
P_{oct} &= 1 - \left(\frac{k_{ue}}{k_t+k_{ue}}\right)^N \left(\frac{k_d}{k_t+k_d}+\frac{k_t}{k_t+k_d}\cdot\left(\frac{k_{ue}}{k_t+k_{ue}+k_d}\right)^{N_T-N}\right), \\
P_{bare} &= \left(\frac{k_{ue}}{k_{ue}+k_t}\right)^{N_T},
\end{aligned}
$$

where N is the number of base pairs unwrapped that allow octamer transfer but not dimer dissociation, and where we assume the competition happens at every base transcribed, along the entire length of the nucleosome, which contains $N_T = 147$ base pairs of wrapped DNA[33] (see Supplementary Discussion 2 for derivation). According to these expressions, as the overall elongation rate through the nucleosome (k_{ue}) becomes larger, the probability of complete histone removal and the resulting production of bare DNA (P_{bare}) should increase monotonically, while the production of transferred octamers (P_{oct}) should instead decrease monotonically. Significantly, this model predicts that the probability of hexamer formation should increase when elongation from low to moderate Pol II elongation rates because as the rate of elongation-dependent octamer unwrapping increases, the probability of histone dissociation effectively competes with that of octamer transfer, enhancing the production of

hexamers as observed in this study (first term in P_{hex} dominates). However, as the rate of elongation and nucleosome unwrapping increases further, the rate of histone dissociation is outcompeted by the rate of complete histone removal and the production of hexamers should attain a maximum and eventually decrease (the last term in P_{hex} dominates).

To test this model, we sought to determine the rates involved in this process. The net rate of nucleosome unwrapping during elongation is equal to the average overall velocity of transcription through the nucleosome (including pausing due to backtracking), which at saturating NTPs (1000 μM) is $k_{ue} = 1$ bp s^{-1} (ref. [17]). Since the average K_m of Pol II for NTP hydrolysis is 100 μM (Supplementary Fig. 3 and Supplementary Discussion 3), we can use Michaelis-Menten kinetics to estimate the net rates of transcription through the nucleosome at 200 μM and 100 μM NTPs; this analysis yields about 0.7 bp s^{-1} and 0.5 bp s^{-1} for these two rates, respectively. Finally, to determine the rate of H2A–H2B dimer loss for pre-assembled octamers directly exposed to the salt concentration used in these studies (300 mM KCl), we performed an ensemble FRET-based assay with fluorescently labeled H2B and H4[31]. These experiments gave $k_d = 0.027 \pm 0.007$ s^{-1} (Supplementary Fig. 4 and Supplementary Discussion 4).

Fit of the experimental data (Fig. 6b) shows that this simple competition model captures correctly the details of hexamer and octamer transfer probabilities, as well as that of complete histone removal, if the initial DNA unwrapped region allowing only octamer transfer (but no dimer dissociation) is $N = 40 \pm 5$ bp and the rate of histone transfer is $k_t = 0.02 \pm 0.005$ s^{-1}. Notice that the value of N obtained here is consistent with the amount of DNA contacted by the H2A–H2B dimer (~30 bp[33]). Therefore, we predict that histone or DNA modifications that destabilize the wrapping of this 40 bp region would favour hexamer formation. The rate of histone transfer is slow and similar to that of dimer dissociation. For histone transfer, the rate-limiting process is most likely the actual hand-off of the histones from the downstream to the upstream DNA, as looping resulting from DNA bending fluctuations is known to be much faster[34].

Note that our mathematical model of histone transfer takes into consideration the increased probability of pausing due to backtracking at low NTPs concentrations and its effect on the overall transcription rate (Supplementary Discussion 3). However, in addition to slowing down the overall elongation rates, extensive backtracking may allow the upstream DNA to rotate so as to face towards the unwrapped histone core, facilitating histone transfer further[18].

DISCUSSION

Our results support a model where nucleosome unwrapping during elongation exposes the histones so that they dissociate from the core octamer unless they interact with another segment of DNA. We propose that because Pol II sharply bends the DNA, it positions the exposed histones very close to the DNA immediately upstream of the polymerase, thus mediating histone transfer to the same DNA molecule via looping. This positioning hypothesis has been proposed before[18,19] and explains both the small size of the loops that allow histone transfer by bridging upstream and downstream DNA, and the small upstream

shift in the position of transcribed nucleosomes. Note that our observation that only a minority of nucleosomes change position after transcription may be influenced by our use of a strong NPS that could bias the histones to transfer and rewrap at the same location as before transcription. The total percentage of shifted nucleosomal particles (hexamer and octamers together) decrease slightly at the NTP concentration is lowered (Supplementary Table 2). Presumably, the slower transcription is more likely to allow the histones to equilibrate on their original position during rewrapping.

In this model of nucleosomal transcription, faster transcription leads to faster overall nucleosome unwrapping, favouring histone dissociation. However, other factors affecting the rewrapping of the histones could influence the outcome of the competition. For example, a trailing polymerase blocking access of the unwrapped histones to upstream DNA[15,35] and histone mutations that destabilize histone-DNA wrapping[36] were both shown to inhibit histone transfer and to promote histone dissociation *in vitro*, as expected according to our model.

This competition model also explains why faster polymerases produce a mix of octamers and bare DNA but yield little or no hexamers upon transcription. For instance, *in vitro,* the majority of Pol III complexes complete transcription through a nucleosome in approximately 30 seconds[12] (Fig. 6b, vertical black line), so we predict that octamer transfer is likely – approximately 40%, while bare DNA production should be about 50%. To obtain hexasomes, on the other hand, two slow processes have to occur before Pol III can finish transcription: dimer dissociation and histone transfer, making the probability of hexamer transfer very unlikely, approximately 10%, under these fast transcription conditions. Note that here we only consider histone transfer within the same DNA molecule and do not include the probability of histone rebinding to other DNA molecules after complete dissociation. These predictions match previous experimental studies with Pol III reporting ~50% octamers and ~50% bare DNA[12] in the presence of competitor DNA, when only transfer in cis is measured.

Moreover, *in vitro* transcription by the even faster SP6 polymerase also leads to the formation of octamers and bare DNA, without hexamer formation, with the percentage of bare DNA increasing as the speed of elongation is increased[11]. Since we estimate that SP6 RNAP is faster than 5bp s^{-1}, our model predicts that the outcome of transcription should be dominated by bare DNA. This prediction might seem contradictory at first with the experimental results, where much lower levels of bare DNA were observed, especially in the absence of competitor DNA[11]. However, our model only considers transfer of the histones in cis (within the same DNA molecule). While for Pol II this is the prevalent scenario, we believe that for faster polymerases (such as SP6) a lot of the histone transfer happens in trans. Since Pol II moves slower, the histones have time to equilibrate with the DNA upstream (which is at a higher local concentration than other pieces of DNA). In contrast, for faster polymerases, the histone octamer detaches quickly, and since it's floating free in solution, it is now just as likely to bind to any piece of DNA (in cis or trans). This interpretation is supported by the observation that for the SP6 polymerase, addition of competitor DNA to the reaction increases the amount of bare transcribed DNA[11]. Moreover, it appears that transfer in trans is seen at higher NTPs concentrations, while at lower NTPs,

transfer in cis dominates, in agreement with our model. However, a quantitative comparison with data obtained for the SP6 RNAP is difficult, since there are multiple – and unknown – transcription rounds for each DNA molecule, leading to a higher probability of complete histone dissociation than described by our model. In addition, since we propose that the geometry of the elongation complex influences histone transfer, we expect that polymerases of significantly different sizes and structures could lead to different positions distributions and transfer probabilities of the transcribed nucleosomes; the importance of these effects remains to be tested.

Gene regulation *in vivo* may result from the modification of any one of the competing rates involved in elongation on a nucleosomal template. While we use a DNA sequence with a higher affinity for the nucleosome than other naturally occurring sequences, we predict that transcription through a weaker nucleosome is faster (an increased k_{ue} rate, because of higher probability of finding the nucleosome locally unwrapped[17]) and the transfer probability decreases (because of lowered rewrapping rates of histones to the upstream DNA). Both these effects would result in higher percentage of bare DNA and hexasomes formations after transcription of weaker positioning sequences. More importantly, elongation factors that increase the net transcription rate of Pol II through the nucleosome would result in an increased probability of complete histone removal from DNA. Alternatively, dimer dissociation from the partially unwrapped octamer could be faster for certain histone variants of H2A[37] or in the presence of histone chaperones that bind the dimer, increasing the probability of hexasome formation, as has been shown *in vitro*[38]. Such transcription-induced alterations in chromatin structure may affect gene expression *in vivo* by reducing or eliminating nucleosomal barriers for future transcription elongation events in a similar manner to results obtained *in vitro*[15,18], or by altering the accessibility of transcription factor binding sites[39]. Finally, we point out that the findings communicated here might also be relevant to other processes that involve advancement of molecular motors through DNA wrapped in nucleosomes, such as processive DNA replication and chromatin remodeling[40].

METHODS

Proteins and DNA purification

His-tagged RNA polymerase II (*S. cerevisiae*, unphosphorylated C-terminal domain) was purified as previously described[41]. The 574 bp DNA template was prepared by PCR from a modified pUC19 plasmid[42] containing the 601 nucleosome positioning sequence[26]. Octamers were reconstituted from recombinant yeast histones[32] and loaded onto the template DNA using salt dialysis[43].

Assembly of elongation complexes with nucleosomes

Pol II was assembled on DNA using the same method and sequences as previously described[17], and the resulting TECs were ligated to downstream DNA containing a pre-loaded nucleosome (Supplementary Fig. 1). The assembly and ligation were performed in TB40 (20 mM HEPES pH 7.8, 40 mM KCl, 10 mM MgCl$_2$, 10 μM ZnCl$_2$, 1 mM β-mercaptoethanol). Transcription was performed at 25 °C in TB300 (the same as TB40,

except with 300 mM KCl instead of 40 mM KCl), using 1 mM of each nucleotide triphosphate (NTP) - unless otherwise specified - and 1 μM pyrophosphate, for 30 minutes.

In the sample used for calculating the position of untranscribed nucleosomes (Fig. 2, blue), the 93 bp double stranded DNA (Integrated DNA Technologies) was ligated in excess to the 574 bp nucleosomal DNA in the absence of Pol II.

Sample preparation for AFM and imaging

Following transcription, samples were fixed by incubating with 1% (w/v) formaldehyde for 2 hours at room temperature. We removed the formaldehyde by dialyzing in TB40 for 45 minutes at room temperature. For deposition, the samples were diluted in TB40 to 2 nM DNA concentrations, placed on freshly cleaved ultra-clean mica (Grade V, Ted Pella, Inc.), and incubated at room temperature for about 2 minutes. The mica discs were then rinsed with purified 18.2 MΩ deionized water and dried using a gentle N_2 gas flow, perpendicular to the mica surface.

AFM measurements were performed with a Multimode AFM Nanoscope V (Veeco Instruments Inc.) equipped with a type E-scanner (10-micron × 10-micron × 2.5-micron vertical range). The samples were imaged in tapping mode using a commercial silicon cantilevers (Nanosensors), with a high-resonance frequency in the range of 260–410 kHz and a spring constant of 46 N m^{-1}. Images (512 × 512 pixels) were captured in the trace direction, at a scan size of 1.5 μm, with a scan rate of 1.5 Hz. The imaging amplitude (amplitude set-point) of the cantilever was maintained by the feedback circuitry to 80–85% of the free oscillation amplitude and the scan angle was maintained at 0. All samples were measured at room temperature in air, at a relative humidity 30%.

Image analysis

Image processing and data analysis were performed using Matlab (MathWorks), with software based on ALEX[27,44]. We imported the images into Matlab, automatically masked all the points higher than 9 nm, and flattened the images by subtracting from each line a polynomial of degree 2 fit to that line. We then identified all the objects higher than 0.2 nm in this flattened image, used these points as a mask, and performed a new line by line flattening on the original with a polynomial of degree 4.

For each complex, the DNA path (passing through the proteins) was digitized and fit by a polynomial of degree 3 (ref. [44]). The polymerase and nucleosome center positions were recorded as the centers of the highest and second highest Gaussians respectively along the identified DNA path (Fig. 1c). The nucleosome heights were measured as the maximum height in a 4-nm box centered at the position of the nucleosome, to correct for cases when the DNA path does not pass through the center of the nucleosome. To account for small height variation among different depositions, we corrected the height of transcribed nucleosomes using the height of untranscribed nucleosomes as a standard. In the chased samples, we first identified the molecules that have Pol II but where Pol II has not yet crossed the nucleosome (as shown in Fig. 1d). We fit the heights of these untranscribed nucleosomes with a Gaussian function for each sample (different tip and deposition), and shift all these distributions such that the Gaussian peak of each one is at 3 nm (which is the

height we get when imaging nucleosomes alone with high frequency tips). Finally, we shift all the other nucleosome heights in that sample by exactly the same amount as the untranscribed nucleosomes. In general, this correction shift is between 0.2–0.8 nm for each sample, with the higher shifts for samples imaged with low frequency tips.

Percentages of hexamers were calculated as the fraction of transcribed particles with heights under 2.4 nm. Amounts of bare DNA are estimated as the percentage of molecules without nucleosomes in the chased samples minus the corresponding percentage in the stalled samples.

Throughout the main manuscript, we use the length of free DNA (DNA not covered by protein) to estimate the position of the nucleosome on the template. Before Pol II passes the nucleosome, it covers part the upstream arm of the nucleosome; in contrast, after transcription, the upstream arm of the nucleosome is completely unobscured. Therefore, in order to obtain an accurate measurement of the length of the upstream arm of the nucleosome before transcription, we imaged a sample that lacks the polymerase, but has a full length template containing the nucleosome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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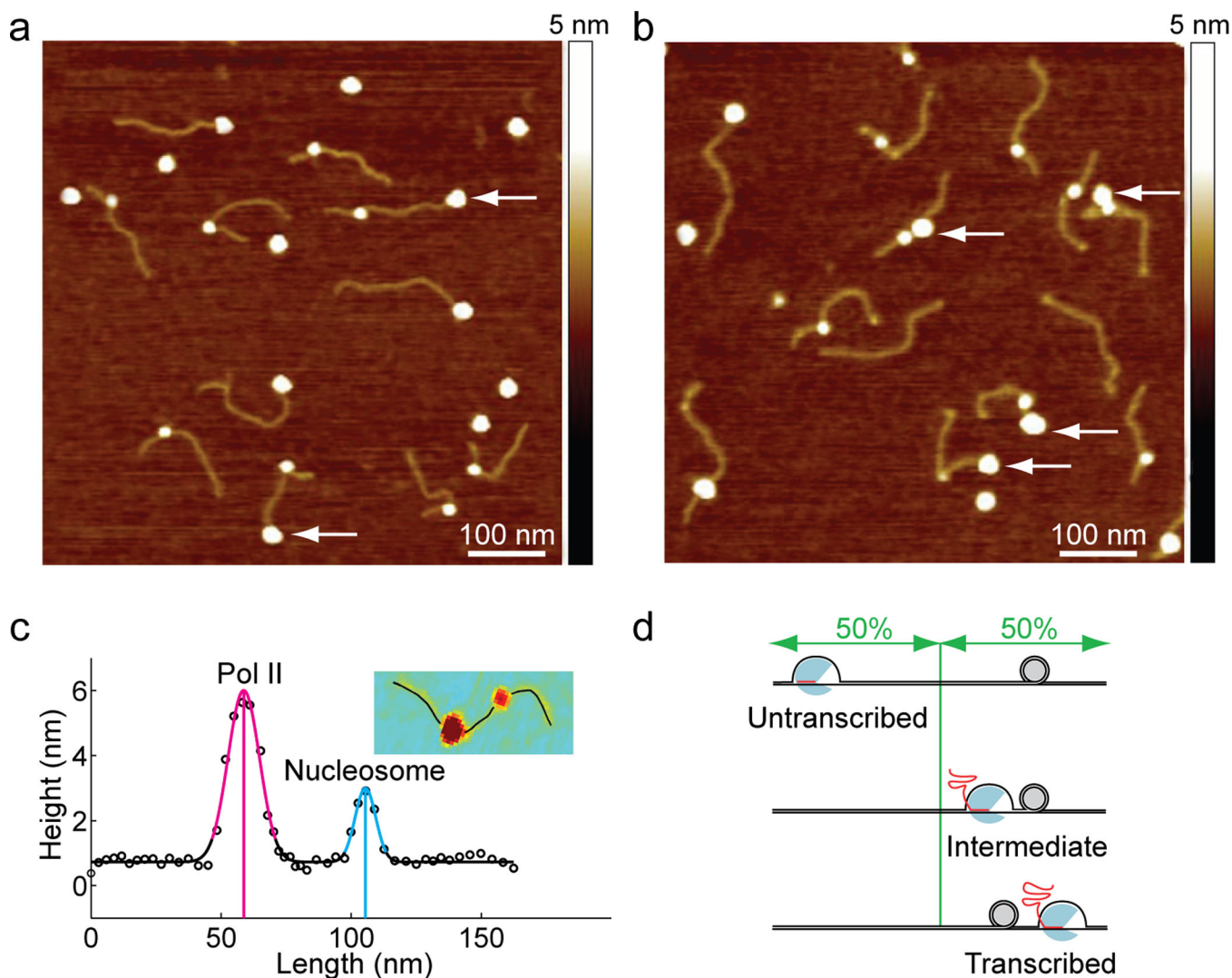
Figure 1.
Snapshots of transcription. AFM images of (**a**) stalled (no NTPs added) and (**b**) chased complexes (all four NTPs added). Only complexes that contain both the polymerase and nucleosome are included for analysis (unless otherwise specified); white arrows indicate Pol II in these complexes. (**c**) The height profile of an example complex (inset) is plotted along the DNA path as black circles. The Pol II and nucleosome heights are fit to Gaussians shown in purple and blue respectively. The free DNA segment lengths (black part of the fit) are defined as the lengths of the paths that start two standard deviations away from the centers of the proteins. (**d**) Schematic of the algorithm used to identify Pol II-nucleosome complexes. When Pol II (blue) is on the long arm of the nucleosome (gray) and its center has not yet reached the middle of the DNA template (green vertical line), we deem the complex untranscribed. When Pol II has passed the middle line of the template, and the Pol II and nucleosome edges are within 5 nm or less of each other, the complexes are tagged as intermediate. When Pol II is on the short arm of the nucleosome, the complex is tagged as transcribed.
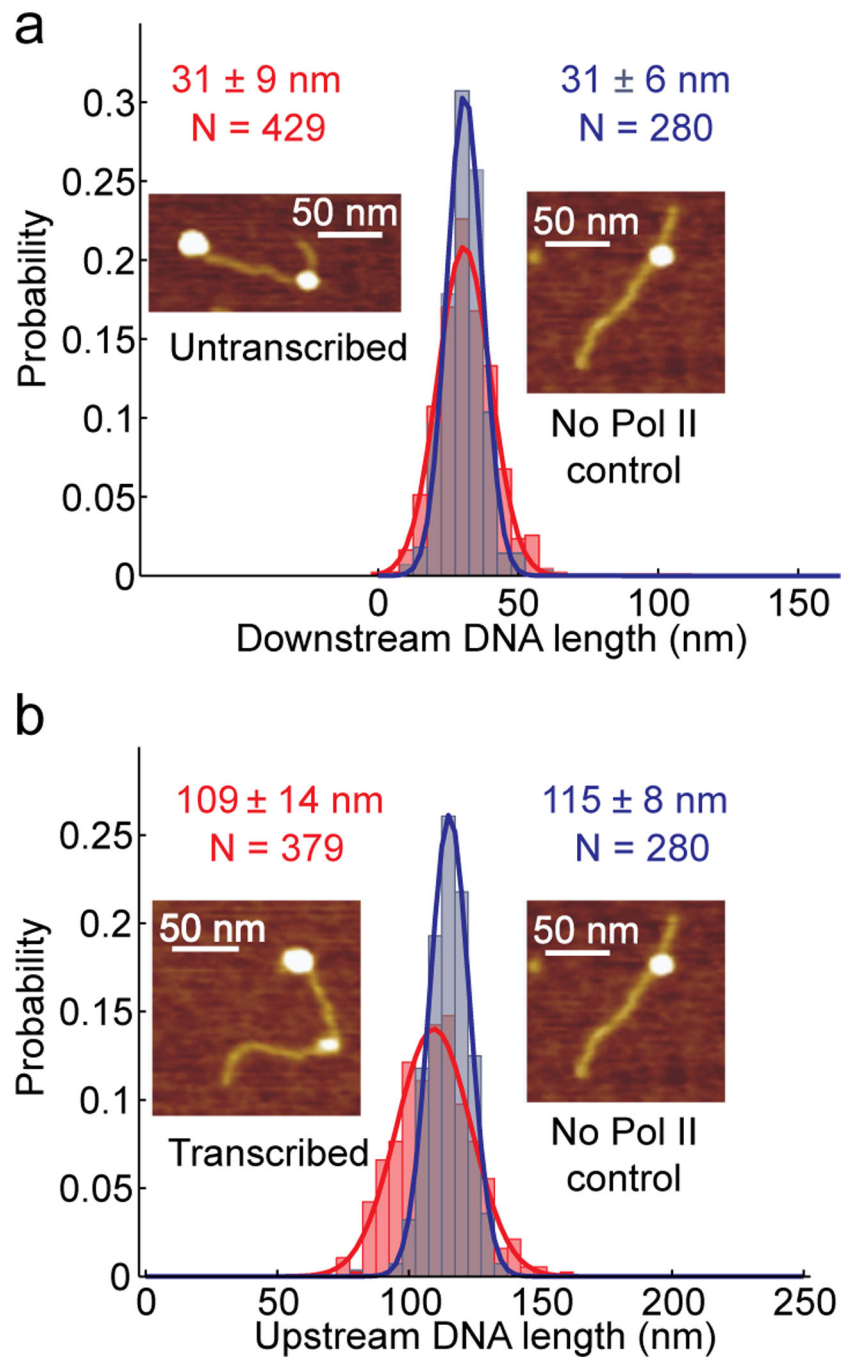
Figure 2.
Nucleosome position. (**a**) The length of the downstream free DNA segments for untranscribed nucleosomes (red) and complexes without Pol II (blue). (**b**) The length of the upstream free DNA segment for transcribed nucleosomes (red) and complexes without Pol II (blue).
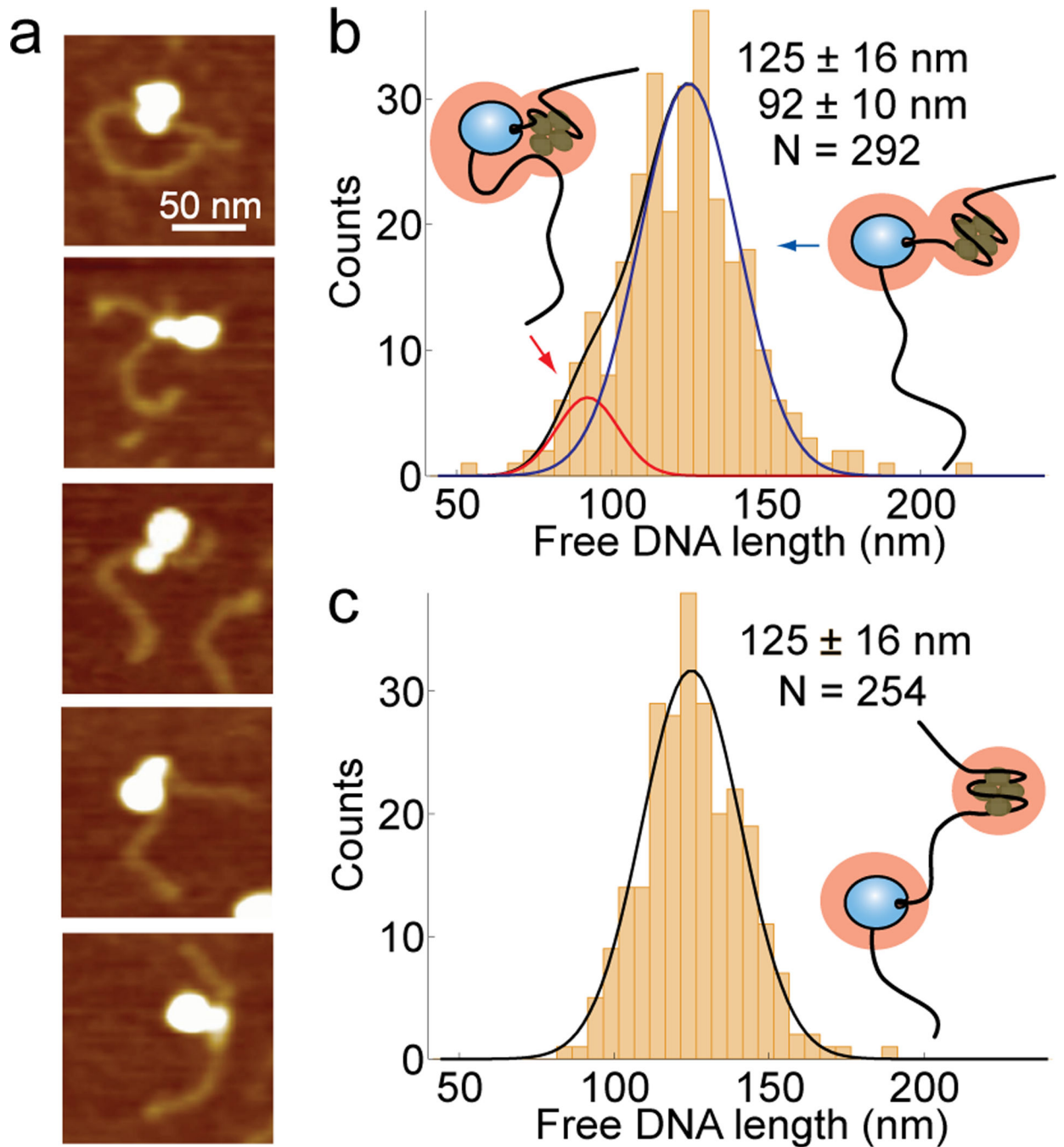
Figure 3.
DNA looping during histone transfer. (**a**) AFM images of complexes where the histones contact both the upstream and downstream DNA. (**b**) Free DNA length in the presence of NTPs (all concentrations) in complexes where Pol II is in the process of transcribing the nucleosome (the length of DNA between Pol II and the nucleosome is less than 5 nm apart) and (**c**) complexes where Pol II has started transcription, but has not yet reached the nucleosome. Insets show the presumed structures of each population, with Pol II in blue, the DNA in black, and the histones in brown; the pink shading reflects the apparent broadening

of the molecules due to the geometry and size of the AFM tip. Numbers indicate the mean and standard deviations of the total free DNA lengths.
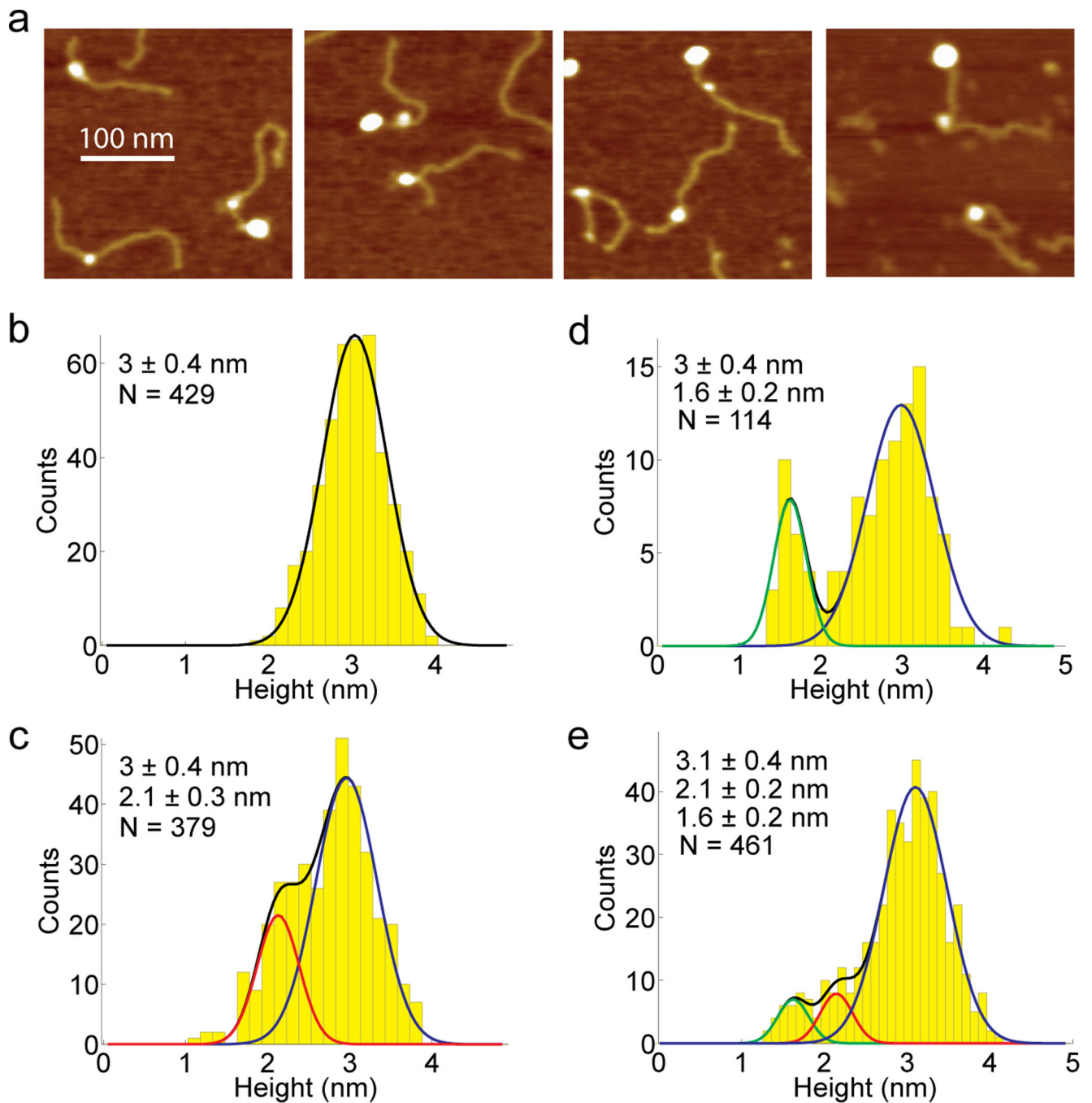
Figure 4.

Transcription leads to hexamer formation. (**a**) Images illustrating change in nucleosome reduction upon transcription. The nucleosomes with reduced height are shown next to normal-sized nucleosomes for comparison. Histograms of nucleosome heights for (**b**) untranscribed nucleosomes and (**c**) transcribed nucleosomes at all NTPs concentrations show the appearance of subnucleosomal particle with reduced height (fit by the red curve). (**d**) Heights of octamers (blue fit) compared with tetramers (green fit). (**e**) Height of

nucleosomes destabilized by incubation in high salt (1 M KCl). We identify the three peaks as tetramers (green), hexamers (red) and octamers (blue).
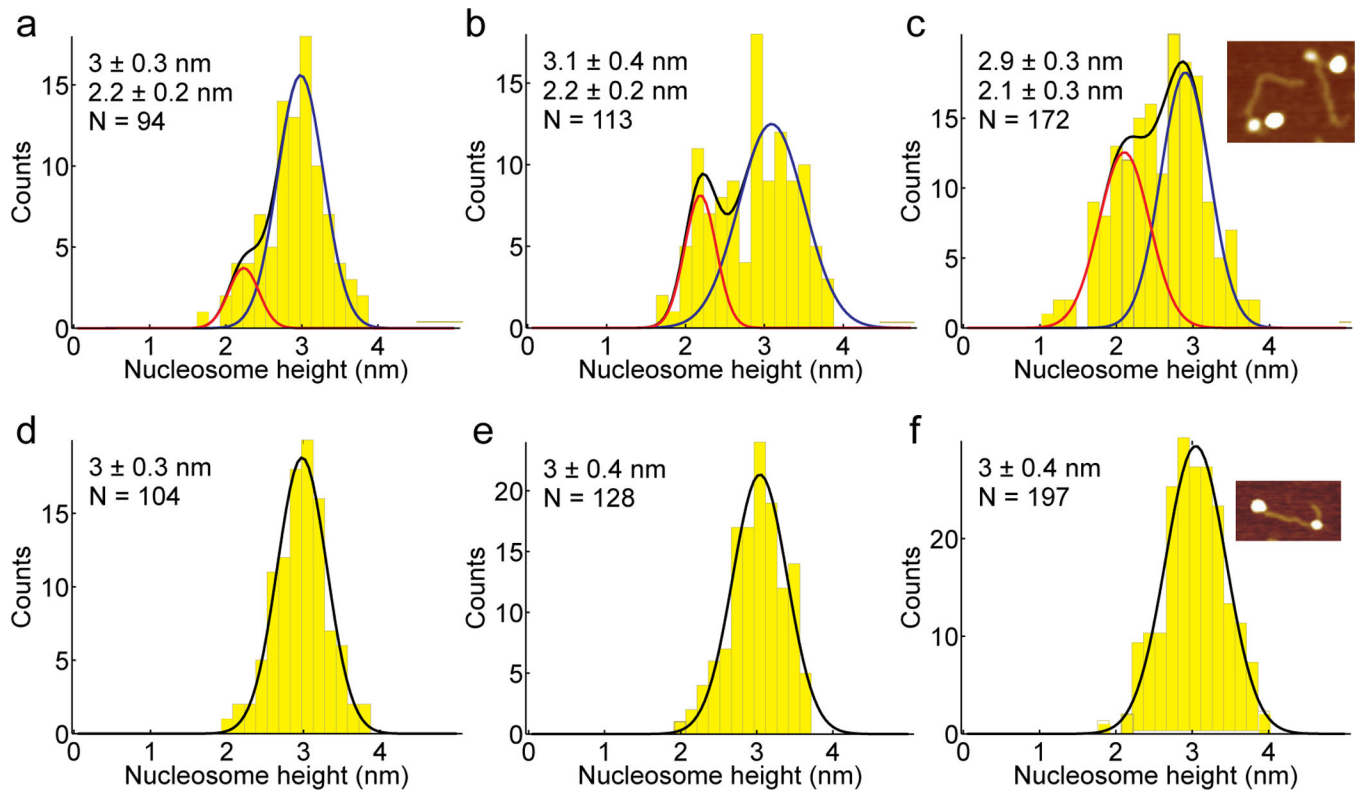
Figure 5.
Histone transfer outcome depends on the speed of transcription. Heights of transcribed nucleosomes at (**a**) 100 μM NTPs, (**b**) 200 μM NTPs, and (**c**) 1000 μM NTPs. Nucleosome heights for molecules where Pol II has not passed the nucleosome in (**d**) 100 μM NTPs, (**b**) 200 μM NTPs, and (**c**) 1000 μM NTPs. The continuous curves represent Gaussian fits to the data. Insets are showing transcribed nucleosomes (top) and untranscribed nucleosomes (bottom).
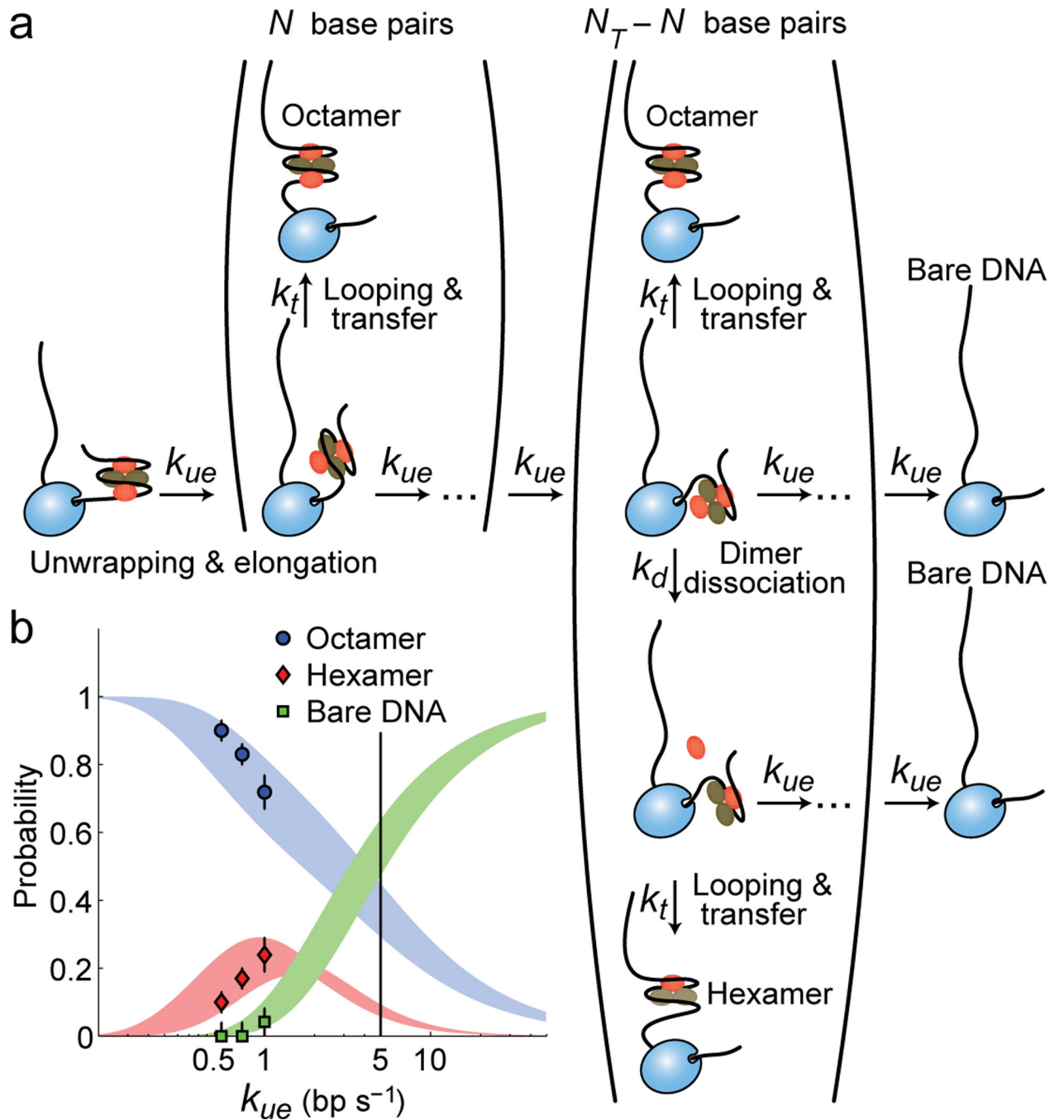
Figure 6.
Histone transfer model. (**a**) Kinetic scheme of transcription and histone transfer. Pol II in blue, H2A–H2B dimers in red, H3–H4 dimers in brown, DNA in black. (**b**) Hexamer (red) and octamer (blue) transfer probabilities, as well as bare DNA formation (green) are plotted as a function of the net nucleosome unwrapping rate during elongation (k_{ue}). Experimental data are shown as circles; the shaded areas represent the model predictions, with the width

reflecting uncertainties in k_t and N, as indicated in the text. The black line marks the elongation rate for the faster polymerase Pol III.