DNA looping mediates nucleosome transfer

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Proper cell function requires preservation of the spatial organization of chromatin modifications. Maintenance of this epigenetic landscape necessitates the transfer of parental nucleosomes to newly replicated DNA, a process that is stringently regulated and intrinsically linked to replication fork dynamics. This creates a formidable setting from which to isolate the central mechanism of transfer. Here we utilized a minimal experimental system to track the fate of a single nucleosome following its displacement, and examined whether DNA mechanics itself, in the absence of any chaperones or assembly factors, may serve as a platform for the transfer process. We found that the nucleosome is passively transferred to available dsDNA as predicted by a simple physical model of DNA loop formation. These results demonstrate a fundamental role for DNA mechanics in mediating nucleosome transfer and preserving epigenetic integrity during replication.

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At the replication fork, a complex interplay of proteins and DNA mediates the faithful duplication of DNA sequence and the subsequent packaging of nascent DNA into chromatin. The spatial organization of epigenetic chromatin modifications is maintained throughout multiple rounds of cell division by the inheritance of parental nucleosomes and the ensuing duplication of covalent modifications to nascent nucleosomes. Parental nucleosomes must be quickly moved from ahead of the progressing replisome to the newly replicated DNA to shuttle parental histones and deposit nascent histones to newly replicated DNA. However, this theory is only loosely described and neglects the role of DNA itself, the fundamental component of nucleosome organization and dynamics. The complex, dynamic environment within which nucleosome transfer occurs is a challenging setting to define essential roles of the individual components that underlie the transfer mechanism. To examine the role of DNA mechanics and identify the minimum system requirements for successful transfer, we utilized a DNA template with a single nucleosome and incrementally added complexity by first displacing the nucleosome mechanically, then with an isolated replicative helicase, and finally with a simplified replication complex. This process allowed us to quantify a fundamental aspect of nucleosome transfer and establish a critical role for the physical properties of DNA during chromatin replication.

**Results**

**Passive nucleosome transfer after mechanical displacement.** In a solution with no free histones, a double-stranded DNA (dsDNA) template, containing a single positioned nucleosome, was mechanically unzipped using an optical trap (Fig. 1a, Supplementary Figs 1A and 2A, and Supplementary Tables 1 and 2). The resulting unzipping force served as a sensitive detector for the presence of the nucleosome, with a force rise above the naked DNA baseline indicating both nucleosome location and composition (Fig. 1b). During unzipping, the force first followed that of naked DNA, until the fork reached the positioning sequence, and then a marked force rise characteristic of a canonical nucleosome occurred, followed by a force drop. As unzipping continued, additional distinct force signatures emerged along the downstream DNA (in front of the moving fork), indicating a re-association of the displaced histone. When the same construct was unzipped in the opposite direction, no force rise occurred until the nucleosome-positioning sequence, illustrating the mono-nucleosome nature of the template (Supplementary Fig. 3). We therefore attribute the subsequent force signatures to the transfer of the original nucleosome. The vast majority of unzipping traces (99%) showed at least one transfer event. For the first such transfer event on each unzipped template (Supplementary Table 2), 67% of traces showed a force signature consistent with that of a nucleosome and 32% were consistent with that of a tetrasome, though it is possible that some of these may have been hexosomes. These results are in agreement with previous findings that parental H3/H4 tetrasomes generally remain intact after replication fork passage, while H2A/H2B dimers are more labile.

**Nucleosome transfer is consistent with DNA loop formation.** Nucleosome transfer in this experimental system could occur via a diffusion-based process, during which histones dissociate from the DNA after being displaced by the fork, diffuse in solution, and then re-associate with another DNA segment. However, this mechanism would result in a distribution of transfer distance that peaks at zero, because histones would most likely associate with a DNA segment in close proximity, such that short distance transfer dominates. In contrast, our data of the first transfer distance peaked at 500–700 bp, and do not support histone dissociation and diffusion. Previous studies also provide evidence indicating that during replication, parental histones are not released into solution, arguing against a diffusion-based mechanism.
An alternative mechanism for nucleosome transfer is based on DNA looping. On fork invasion of the nucleosome, the histone surfaces become partially exposed and available dsDNA may loop back onto the histone surfaces and capture the histones. As the fork progresses, the nucleosome is thereby repositioned to another location on the DNA. Although this possibility was raised nearly 20 years ago, there has been no direct experimental evidence to date. Importantly, support for such a model must be made quantitatively, because DNA loop formation makes explicit predictions on the loop size distribution and thus the nucleosome transfer distance distribution. Transfer distances below 200 bp are energetically unfavourable as the persistence length of DNA is ~150 bp, thus prohibiting the formation of small loops. Very long transfer distances are also improbable, because the putative acceptor DNA must sample a large volume, reducing its chance of encountering the initial nucleosome. Consequently, the defining features of the DNA looping model are an extremely low transfer probability at short distances, a sharp rise in the probability at ~200 bp followed by a peak at ~500 bp and a long tail.

Figure 1c shows a comparison of measured transfer distances and a direct prediction by the loop formation theory (not a fit). There is a good agreement between the two distributions (Methods; Supplementary Fig. 4A). The loop formation model depends predominantly on the persistence length of DNA, which dictates the likelihood of downstream DNA being in close proximity to the nucleosome. Although persistence length is DNA sequence-dependent, such dependence should be secondary on the length scale considered here. Consistent with this, an additional experiment conducted with a DNA template of a different sequence yielded a similar transfer distance distribution (Supplementary Fig. 5). In addition, we found that increasing the rate of unzipping 10-fold does not lead to major changes in the transfer distance distribution or efficiency, although there is a slight increase in the nucleosome fraction (Supplementary Fig. 6 and Supplementary Table 2b).

Local DNA concentration and DNA elasticity dictate transfer. To further examine whether DNA looping mediates nucleosome transfer, we carried out nucleosome disruption experiments in the presence of competitor DNA (Fig. 2a), which should compete with the downstream DNA for acceptance of a transferred nucleosome. The DNA looping model directly predicts an effective local concentration of the available downstream DNA at the nucleosome (Methods), and thus nucleosome transfer to downstream DNA should decrease with an increase in DNA concentration. For example, we showed that increasing the competitor DNA concentration 10-fold does not lead to major changes in the transfer distance distribution or efficiency, although there is a slight increase in the nucleosome fraction (Supplementary Fig. 6 and Supplementary Table 2b).

Figure 2 | Mechanical displacement of a single nucleosome in the presence of competitor DNA. (a) Experimental configuration. A single dsDNA molecule was mechanically unzipped using an optical trap (Supplementary Fig. 1A). The dsDNA contained a positioned nucleosome followed by a long naked DNA segment (Supplementary Fig. 2A, Supplementary Table 1 and Methods). Linear competitor dsDNA of 2,987 bp was introduced into the chamber at varying concentrations immediately before mechanical disruption. N = 121, 52, 57, 39, 38 and 92 traces for 0, 30, 50, 100, 200 and 300 ng µl−1 competitor DNA concentrations, respectively. (b) Two example unzipping traces in the presence of 100 ng µl−1 of competitor DNA. The top trace shows an absence of a transferred nucleosome to the downstream DNA, whereas the bottom trace shows the presence of a transferred nucleosome. (c) The probability of nucleosome transfer to downstream dsDNA as a function of competitor DNA concentration. Error bars represent 95% confidence intervals (Methods). A direct prediction (not a fit) based on DNA looping and a simple competitive binding relation (Methods) is shown for comparison.
competitor DNA concentration, following a simple competitive binding relation (Methods).

In these experiments, nucleosomes were disrupted in the presence of varying concentrations of competitor DNA that was of nearly equal length as that of the downstream DNA. As expected, with an increase in competitor DNA concentration, the probability for nucleosome transfer to the downstream DNA decreased. Example trace without and with transfer to the downstream DNA are shown in Fig. 2b. Figure 2c shows a summary of the probability of transfer to the downstream DNA as a function of competitor DNA concentration, along with a direct prediction (not a fit) based on a simple competitive binding relation. There is good agreement between measurements and prediction at competitor DNA concentrations ≤100 ng μl−1; concentrations above this threshold resulted in measured values somewhat larger than predicted. Deviation in this range is likely due to the use of a simple competitive binding relation without consideration of the excluded volume effect (Methods), which becomes significant at high competitor DNA concentrations resulting in a preference for intra-DNA transfer. Therefore, over the range where the competitive binding relation holds, these results support DNA loop formation as the mechanism of nucleosome transfer.

**Helicase-induced nucleosome transfer.** Although fork progression was initially carried out mechanically, in vivoin vivo it is mediated by helicases that unwind dsDNA during replication. We therefore used T7 helicase as a simple model system to investigate the fate of a single nucleosome located on a parental dsDNA template during unwinding (Fig. 3a, Supplementary Figs 1B and 2A, and Supplementary Table 1). As shown in Fig. 3b, before encountering the nucleosome, the helicase unwound the dsDNA at the expected rate, as reported previously28−32. On encountering the nucleosome, the helicase showed a discrete pause, consistent with previous studies that characterized nucleosomes as major barriers for helicase unwinding33. Initial pausing occurred near the dyad region of the positioned nucleosome, which contains the strongest histone–DNA interactions10−12,15,16. In 89% of the traces, helicase eventually exited the pause within the experimental time window of 150 s, and then proceeded at its initial speed, indicating the complete displacement of the nucleosome. As the helicase unwound further along the DNA, it paused again at locations initially lacking nucleosomes. These additional pauses suggest nucleosome transfer downstream from its original location. Analysis of the distance of the first transfer event revealed a distribution that was again in agreement with prediction by the DNA loop formation model (Methods; Supplementary Fig. 4B). Thus, a simple passive mechanism is able to account for nucleosome transfer during fork progression, carried out either mechanically or by a motor protein.

**Nucleosome transfer to leading strand at replication fork.** During DNA replication, dsDNA available for nucleosome transfer is located behind the replication fork on the nascent daughter duplexes, which are poised to accept parental nucleosomes from ahead of the replication fork22,34,35. We hypothesize that if nucleosome transfer is dictated by DNA loop formation, transfer should take place on the upstream dsDNA, in a similar manner as demonstrated for the downstream dsDNA. To investigate this hypothesis, we carried out leading strand replication using the T7 replisome to generate upstream dsDNA. The parental DNA template contained a single nucleosome with minimal naked DNA downstream (ahead) of the nucleosome (Fig. 4a, Supplementary Fig. 2B and Supplementary Table 1). To quantitatively assay the position of the transferred nucleosome, the 5′-end of the replicated leading strand was fluorescently labelled, and the replication product was subjected to exonuclease III digestion before being assayed by a denaturing gel (Fig. 4b, replicates shown in Supplementary Fig. 7). The single-stranded DNA (ssDNA) resistant to digestion provided a quantitative measure for nucleosome position following DNA replication. The resulting distribution of the ssDNA lengths shows nucleosome transfer, peaked at 500–700 bp upstream of the initial nucleosome position. Although the measured transfer distance showed some sequence preference not accounted for by the loop formation model in its current simplest form, the overall features of the
distribution are consistent with the model. Furthermore, the measured effect of competitor DNA on nucleosome transfer in these bulk replication assays is again well predicted by the DNA looping theory (Supplementary Fig. 8).

**Discussion**

Taken together, results from these three distinct experimental approaches provide consistent support for passive nucleosome transfer by DNA loop formation (Fig. 5a). As a nucleosome is displaced, it will be spontaneously transferred to available dsDNA, and this transfer is mediated by the formation of a DNA loop that bridges the nucleosome from its initial location to its new location (Fig. 5b). Previous studies found nucleosomes remain associated with DNA during transcription after the passage of RNA polymerase. Earlier studies with pol III suggested a loop of 80 bp (ref. 37); whereas more recent work with pol II favours a ‘zero-size’ DNA loop. In contrast, for DNA replication, such small, or non-existent, loops are not consistent with previously measured in vivo distance scales.

Indeed, a number of in vivo and in vitro chromatin replication studies support key aspects of our looping model. DNA loop formation requires at least ~200 bp of free dsDNA to form a minimal DNA loop, consistent with the 200–600 bp of available naked nascent dsDNA present immediately upstream of the replication fork in vivo. In addition, parental nucleosomes have been shown to be located within ~400 bp of their original positions after the completion of the cell cycle, close to the most probable loop size. DNA loop formation may also be facilitated by the configuration of nascent dsDNA strands as they emerge from the replisome, which would contribute to the partitioning of nucleosomes between the two daughter strands by coordinating nucleosome transfer with DNA synthesis.

*In vivo*, passive transfer would only occur if there is sufficient available dsDNA to accept parental histones. Consistent with this requirement, overexpression of new histones or perturbation of chaperone function both result in replication fork stalling. This implies that when daughter DNA is saturated with new histones, or new histones are not positioned properly, the parental nucleosome at the fork cannot be efficiently transferred and therefore becomes a substantial barrier for replication. Nascent histone deposition is likely coordinated with the transfer of parental nucleosomes, possibly by regulation of the deposition of new histones through a feedback mechanism involving the transfer of parental nucleosomes.

Although our model does not require specific interactions of histones with the replisome, recent studies have shown that histone H3 may interact with the eukaryotic helicase, providing insight into how replisome progression and histone dynamics may be coordinated. However, the action by which this potential intermediate transfers parental histones to the nascent DNA has yet to be elucidated and is still controversial.

The *in vivo* mechanism for nucleosome inheritance likely requires the coordination of many factors acting at, and around, the replication fork. These complex processes can take place on a simple platform dictated by DNA mechanics. The data presented here have quantified the ability of available DNA to facilitate the transfer of parental nucleosomes.

Our proposed model of passive parental nucleosome transfer via DNA loop formation describes a fundamental mechanism to facilitate parental nucleosome transfer while also permitting broader coordination for the deposition of new histones. DNA loop formation thus provides a simple pathway that facilitates cellular complexity by exploiting fundamental physical properties.

**Methods**

**Protein purification.** Histones were purified using hydroxyapatite precipitation from HeLa-S3 cells purchased from the National Cell Culture Center. Nuclei were extracted from a pellet from 61 of cells in Nuclear Pellet Prep Lysis Buffer (20 mM HEPES (pH 7.5), 3 mM MgCl2, 250 mM sucrose, 0.5% (v/v)
The nucleus as indicated in Fig. 3. The prediction (black, not a fit) from the introduced by the fraction of reaction that did not proceed past the strand replication (purple; Supplementary Fig. 7). Note that the peak near measured using three experimental approaches: mechanical fork replication fork, a DNA loop forms in one of the daughter duplexes (red), replisome (purple) encounters a parental nucleosome (green) at the formation.

**Figure 5 | The passive nucleosome transfer model via DNA loop formation.** (a) Comparison of nucleosome transfer distance distributions as measured using three experimental approaches: mechanical fork progression (red; Fig. 1c); helicase unwinding (green; Fig. 2c); and leading strand replication (purple; Supplementary Fig. 7). Note that the peak near zero from the leading strand replication curve (purple) was background introduced by the fraction of reaction that did not proceed past the nucleosome as indicated in Fig. 3. The prediction (black, not a fit) from the DNA looping model is also shown for comparison. (b) A mechanistic model of passive nucleosome transfer mediated by DNA loop formation. When a replisome (purple) encounters a parent nucleosome (green) at the replication fork, a DNA loop forms in one of the daughter duplexes (red), bridging the nucleosome from its initial location to its new location and thus facilitating direct transfer to the daughter duplex. Nascent histones (yellow) are also deposited on the daughter strands by chaperones.

IGEPAL CA-630 (NP-40) nonic detergent, 1 tablet per 50 ml Complete protease inhibitor cocktail (Roche) and 3 mM 2-mercaptoethanol.87 The nuclei pellets were frozen in liquid nitrogen and stored at ~80°C. Core histones were purified using a hydroxyapatite Bio-gel HTP gel (Bio-Rad Laboratories) slurry, according to methods by Wolffe and Ura48, with the omission of MNase digestion before fractionation. Aliquots of purified histones were stored in ~80°C at a final concentration of 2.7 μM.

Nucleosomes were assembled on the Widom 601 nucleosome-positioning element89 by salt dialysis.90-13,16,30,50-51. For the mechanical displacement assay, nucleosomes were assembled on a 764 bp template at a molar ratio of 1:25:1.00 of histone octamer to DNA. For the helicase displacement assay, nucleosomes were assembled on a 250 bp template at a molar ratio of 1:75:1.00 of histone octamer to DNA. Template details can be found in Supplementary Table 1 and example native gels of nucleosome assembly can be found in Supplementary Fig. 9.

Wild-type T7 helicase gpaA was purified from *Escherichia coli*, by the Patel Lab94.

**DNA template construction**. All experiments required the use of forked DNA templates, each of which consisted of two arms and a trunk. These forked DNA structures were prepared by the ligation of DNA adapter oligos (Supplementary Table 1) to a labelled PCR product. The adapter oligos contained a region that annealed to one another thus forming a Y-structure DNA template59. For all single-molecule experiments, each arm was ~1,000 bp (see Supplementary Table 1 for sequences of all DNA segments). For the mechanical displacement experiments,
Samples were then buffer exchanged into 1× NEBuffer 1 (NEB) using Amicon Ultra-0.5 centrifugal filter units with Ultrafree-30 membranes (Millipore). A volume of 400 μl of 1× NEBuffer 1 was added and samples were centrifuged at 5,000g for 5 min at 4 °C four times total. Samples were spun an additional 5 min at 4 °C for the final concentration to reduce the retained volume. Each reaction was then digested with 100 units of exonuclease III (NEB) at 37 °C for 30 min. Samples were precipitated by addition of 500 μl solution containing 0.5% linear polyacrylamide, 7% saturated ammonium acetate and 91% ethanol. Incubated overnight at ~80 °C and centrifuged at 16,000g for 20 min. Samples were then decanted and 500 μl 70% ethanol was added followed by vigorous vortex mixing. This was followed by centrifugation at 16,000g for 10 min. Tubes were then carefully decanted and dried for 5 min in a vacuum chamber. Pellets were resuspended in 20 μl of alkaline gel buffer (5 mM NaOH and 1 mM EDTA) by vortexing and incubated for 30 min at 37 °C. A volume of 4 μl alkaline loading buffer (50% glycerol, 30 mM NaOH and 6 mM EDTA) was then added and each sample was heated at 95 °C for 5 min to denature the DNA, and then placed on ice. Samples were separated on 1% agarose gels in alkaline gel buffer using electrophoresis at 4.7 cm 2/3 h for 4 h, and quantified using a Typhoon imager (GE).

Data were then converted from intensity at each position within a gel scan to probability distributions for nucleosome transfer distance. The Cy5-labelled DNA ladder was created using PCR products of 125, 332, 497, 649, 859, 1,390 and 2,000 bp, so for long DNA we applied the Daniels approximation. For a given DNA length, this fit the log of ladder band length versus gel position to a quadratic function, and then linearly interpolated between ladders run in different lanes. The intensity was fitted as what have been measured, assuming the measurements were from the theoretical distribution. The P values (0.81 for Fig. 1c and 0.95 for Fig. 3c) indicate a strong agreement between the measurements and the DNA looping model. The DNA before conversion to a weight concentration. For the 2,987 bp downstream DNA before conversion to a weight concentration. For the 2,987 bp downstream DNA before conversion to a weight concentration.

DNA loop formation modelling. The DNA looping probability, or the Jacobson–Stockmayer J-factor, was calculated for a dsDNA with a persistence length of 50 nm (refs 62,65) using the worm-like-chain model from equation (50) of DNA loop formation modelling. The DNA looping probability, or the Jacobson–Stockmayer J-factor, was calculated for a dsDNA with a persistence length of 50 nm (refs 62,65) using the worm-like-chain model from equation (50) of DNA loop formation modelling.
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Acknowledgements
We thank members of the Wang laboratory for critical reading of the manuscript.

We especially thank Drs James T. Inman, Shanna L. Moore, Bo Sun, Tung Le, James P. Sethna, Robert M. Fulbright and Martin Wells for helpful discussions and technical advice, and Dr Marcus Smolka and Eric Sigga for commenting on the manuscript. We acknowledge graduate traineeship support to L.D.B. from Cornell University’s Molecular Biophysics Training Grant (T32GM008267), a postdoctoral support to R.A.F. from the American Cancer Society (125126-PF-13-205-01-DMC), support from National Institutes of Health grants (GM059849 to M.D.W. and GM55310 to S.S.P.) and support from National Science Foundation grant (MCB-0820293 to M.D.W.).

Author contributions
L.D.B., R.A.F. and M.D.W. designed the experiments and wrote the manuscript; L.D.B. and R.A.F. performed the mechanical displacement assay; L.D.B. performed the helicase-unwinding assays, supplementary single-molecule experiments and competitor DNA bulk replication assay, and purified the histones; R.A.F. performed the bulk replication assay, the initial DNA looping theoretical calculations and wrote the custom data analysis software; S.S.P. provided the purified T7 gp4 and helped to revise the manuscript; M.D.W. supervised the project.

Additional information

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How to cite this article: Brennan, L. D. et al. DNA looping mediates nucleosome transfer. Nat. Commun. 7, 13337 doi: 10.1038/ncomms13337 (2016).

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