

# Coupling of replisome movement with nucleosome dynamics can contribute to the parent–daughter information transfer

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## ABSTRACT

**Positioning of nucleosomes along the genomic DNA is crucial for many cellular processes that include gene regulation and higher order packaging of chromatin. The question of how nucleosome-positioning information from a parent chromatin gets transferred to the daughter chromatin is highly intriguing. Accounting for experimentally known coupling between replisome movement and nucleosome dynamics, we propose a model that can obtain de novo nucleosome assembly similar to what is observed in recent experiments. Simulating nucleosome dynamics during replication, we argue that short pausing of the replication fork, associated with nucleosome disassembly, can be a event crucial for communicating nucleosome positioning information from parent to daughter. We show that the interplay of timescales between nucleosome disassembly ( $\tau_p$ ) at the replication fork and nucleosome sliding behind the fork ( $\tau_s$ ) can give rise to a rich ‘phase diagram’ having different inherited patterns of nucleosome organization. Our model predicts that only when  $\tau_p \geq \tau_s$  the daughter chromatin can inherit nucleosome positioning of the parent.**

## INTRODUCTION

The fate of a cell is controlled not just by the DNA sequence alone but also by the organization and the kinetics of proteins along the DNA. In most eukaryotes, a huge fraction of the genomic DNA (e.g. >80% in yeast gene regions) is covered by histone proteins leading to formation of a chromatin that appears like a ‘string of beads’ (1,2). Advances made in the last many years have confirmed that nucleosomes and their organization play an important role in nearly all cellular processes. For example, nucleosomes are known to cover

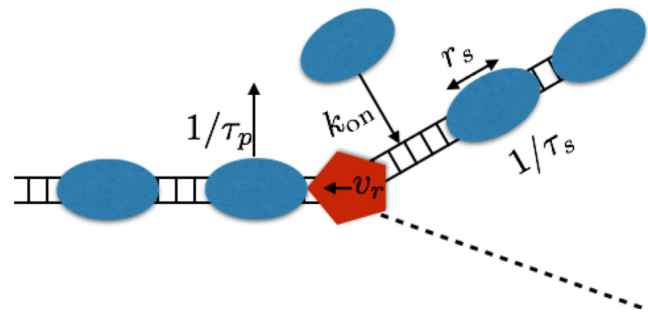
transcription factor binding sites and restrict proteins from accessing those crucial sites along the genome and, hence, regulate gene expression (3–7). There are very different nucleosome organizations in coding regions and promoter regions of genes, indicating the importance of the high diversity in nucleosome organization (3,8–10). Precise nucleosome organization is also crucial for higher order packaging of DNA as the polymorphic chromatin structure depends on linker length distribution (11,12).

Since the precise positioning of nucleosomes is important, the natural question is, how do cells transfer this information about nucleosome positioning from one generation to another? How do daughter cells know about the nature of nucleosome positioning in the parent cells? This is an intriguing question for which we do not know the precise answer. One hypothesis argues that the DNA sequence determines the nucleosome positioning along the genome, and hence, the information is transferred with the DNA (8,13). However, various experiments have indicated that the DNA sequence alone would not determine the nucleosome positioning in the genome (9,14)—ATP-dependent chromatin remodelling, statistical positioning and other factors play equally important role (15–19). Moreover, different cell types (neuronal, muscle, epithelial cells etc) have exactly the same DNA, but they have very different organization of the chromatin, gene expression pattern and function (2). Another major drawback of the sequence-dictated model of self-organization of nucleosomes is that attaining an ‘equilibrium’ (steady state) nucleosome organization may take long time (20), and hence, regulation of genes prior to attaining a desired nucleosome distribution may fail. An alternative hypothesis is that nucleosome positioning needs to be inherited, somehow, during replication so that the daughter cells can appropriately regulate their gene expression in an independent manner (21). This hypothesis is partially strengthened by recent experiments (22) which show that nucleosome positions are conserved at inactive sites behind the replication fork.

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How does the *de novo* nucleosome assembly happen during DNA replication? Experiments have been giving us major insights into the *de novo* nucleosome assembly in the various gene regions (22–33). For example, Lucchini *et al.* have shown that nucleosomes are properly organized shortly after passage of the replication machinery and propose that the nucleosome positioning is the initial step of chromosome maturation (24). Recently, Alabert *et al.* have shown that not just nucleosome positioning, but nucleosome modifications are also inherited from the parent to daughter (25). Additionally, Blythe *et al.* have shown that chromatin accessibility is also conserved throughout the cell cycle (26). Moreover, experiments from different groups over the years have shown that DNA replication is coupled with nucleosome assembly (27–29). In a recent publication (27), Smith and Whitehouse have shown that nascent chromatin plays a role in termination of Okazaki fragment synthesis. This indicates the importance of nucleosome positioning immediately behind the fork during replication. In another paper, Yadav and Whitehouse showed that the nucleosomes behind the replication fork also get repositioned via ATP-dependent chromatin remodeling machines, and such remodeling is essential for obtaining certain features associated to nucleosome organization (30). Requirement of ATP-dependent chromatin remodelling enzymes to reorganize nucleosomes, after replication, is also proposed by Fennessy *et al.* (31). Recently, in yeast, Vassuer *et al.* studied the maturation of nucleosome organization following genome replication (32) and analysed the role of transcription in the maturation of nucleosome organization to their mid log position of active gene region. They showed that soon after replication, in downstream TSS, the nucleosome organization is not proper and it takes time to mature. Ramachandran and Henikoff found that after replication nucleosome occupancy at active gene regions may differ from steady-state pattern owing to the competition between nucleosomes and various regulatory factors that bind DNA (22). However, they also found that nucleosome occupancy in the inactive region is very similar to the nucleosome of the parent chromatin, suggesting that inheritance of nucleosome positioning after replication at certain locations along the genome. There has been hardly any theoretical/computational study investigating the *de novo* nucleosome assembly. To the best of our knowledge, only the work of Osberg *et al.* (34) investigates some aspects of the *de novo* nucleosome assembly. However, they do not address the question of inheritance of precise nucleosome positioning from parent chromatin to the daughter.

In this work, we investigate the nucleosome organization immediately after replication, accounting for various experimentally known facts. We present a kinetic model incorporating replisome (replication fork) movement, nucleosome disassembly ahead of the fork, and nucleosome deposition and repositioning (sliding) of nucleosomes behind the fork. We show that pausing of the fork during disassembly of nucleosomes on parental chromatin and sliding/repositioning of nucleosomes on daughter chromatin behind the fork are crucial events dictating the nucleosome positioning after replication. We systematically explore the parameter space in the model and point out the parameter regime where inheritance of nucleosome positioning may be observed. We



**Figure 1.** Schematic diagram representing our model for nucleosome disassembly/assembly dynamics during replication. The replisome complex (red pentagon) at the fork moves with a velocity  $v_r$  in the direction shown by the arrow. As it encounters a nucleosome (blue oval) ahead, the fork movement pauses for a mean time  $\tau_p$  which is the timescale for the obstructing nucleosome to get disassembled (disassembly rate  $=1/\tau_p$ ). When sufficient length of double stranded DNA ( $\geq 150$  bp) is made, a nucleosome gets assembled behind the fork with an intrinsic rate  $k_{on}$ , and the newly assembled nucleosomes will get slid for a time period of  $\tau_s$  at a rate  $r_s$ . The sliding happens in such a way that the nucleosome will get slid to the middle of the available free region.

also study the competition between nucleosomes and non-histone proteins, and how they affect the nucleosome positioning during replication.

## MATERIALS AND METHODS

### Model for nucleosome kinetics during replication

Here we present a model to study the nucleosome reorganization following gene replication. In this model we start by considering an initial (parental) chromatin—DNA bound with nucleosomes—having a specific nucleosome organization. The DNA is considered as a one-dimensional lattice with each base pair marked with an index  $i$ . The nucleosome is modelled as a hard-core particle sitting on the lattice, occupying a space of  $k = 150$  lattice sites (see Figure 1). At  $t = 0$ , the replisome starts replication process from the replication origin ( $i = 0$ ), and it moves with a bare rate  $v_r$  (rate of fork movement unhindered by nucleosomes) in the forward direction. As the replisome moves forward, it may encounter a nucleosome. Given that the nucleosome is a stable complex, there can be delays in fork progression as the remodeling enzymes try to disassemble the nucleosome ahead of the fork. This delay in fork progression will be referred to as a ‘pause’ event. This is a pause in fork progression and not in other processes. We consider  $\tau_p$  as the typical timescale of this pause event (33,35). In other words,  $1/\tau_p$  is the eviction rate of nucleosomes at the replication fork. The replisome, as it moves, creates new double-stranded DNA (dsDNA) behind it; whenever the length of the newly synthesized dsDNA is larger than the size of a nucleosome ( $> 150$  bp), a new nucleosome can occupy that space with an intrinsic rate of  $k_{on}$ . The effective nucleosome binding rate is proportional to the freely available space ( $\ell_f$ ) on the dsDNA for nucleosomes to bind, i.e.  $k_{on}^{eff} = k_{on} \times \ell_f$  (see Supporting Information (SI)). As the replisome moves further, the process repeats. At this point, it is important to note that, as mentioned earlier, recent experiments have indicated that the nucleosome deposition behind the fork hap-

pens soon after the fork movement (22,25,27) and is crucial for efficient replication (27).

It has also been shown that the newly deposited nucleosomes get slid/repositioned with the help of appropriate ATP-dependent chromatin remodelers, and this is crucial for the formation of proper nucleosome positioning (30). In the model, taking cues from recent experiments (18,19), we assume that a nucleosome gets slid back and forth until it settles down at the middle of the available free dsDNA. To achieve this repositioning, we do the following exercise: each nucleosome has a rate of sliding given by  $r_s = r_{s0}(i - i_0)$  toward the mid position  $i_0$ , from the current location  $i$ , with a step of size 10 bp. Here,  $r_{s0}$  is the intrinsic rate of sliding and  $i_0$  is the mid position of the locally available free (linker) dsDNA at that instant;  $i_0$  will evolve as the nearest nucleosome or the fork is displaced. However, the nucleosome does not slide for ever; it stops sliding after a time  $\tau_s$ . The sliding could stop because the ATPase that facilitates sliding can disassemble or stop functioning after a certain time  $\tau_s$ .

What is given above is our basic model that describes nucleosome assembly dynamics. However, we have also extended the model to introduce binding of non-histone proteins such as gene regulatory factors (GRFs) or proteins that bind near replication origins. These proteins are considered as sterically interacting particles like a nucleosome, but with different sizes and different parameter values. For example, GRFs will have size lesser than a nucleosome while their sliding rate will be zero. Using the same simulation set up we have developed here, we investigate the role of different non-histone protein factors and how they affect nucleosome organization post replication.

**Parameters and their numerical values.** There are five parameters (rates or timescales) in the model. However, many of them are constrained by known experimental data. The bare rate of replication ( $v_r$ ) and the pausing timescale of the replication fork are constrained by the time it takes to complete replication over a stretch. These rates are taken such a way that 1.5–2 kb of dsDNA is replicated in a minute (33). Similarly, nucleosome density of the parent constrains the nucleosome deposition rate, and the fork velocity. In our simulations we have used a nucleosome density range of 60–90% as known *in vivo* (4,8). Apart from the above constraints, various experiments published in the literature also give us relevant ranges of parameter values. The forward movement rate of the replication fork is estimated in the range 10–500 bp/s (36). The binding rate of nucleosomes ( $k_{on}$ ) is estimated to be  $\approx 0.1$ –10 (bp s)<sup>-1</sup> (20,37). The fork pausing timescale,  $\tau_p$ , which happens due to delay in nucleosome disassembly ahead of the fork, can be estimated to many seconds/tens of seconds (20,33,37). This is also comparable to the known timescale of similar pausing during transcription (38,39). In (33), nucleosome disassembly timescale ahead of the replication fork (the pausing timescale) is estimated as 7 s, assuming a uniform disassembly rate everywhere. However, there will be heterogeneity (due to DNA sequence/nucleosome stability) and it may vary over a range  $\sim 7$  s depending on the location/cell-type. Hence we have done our simulations for a wide range of  $\tau_p$  values. We do not know the sliding parameters precisely.

Hence in this work, we vary the sliding duration parameter ( $\tau_s$ ) over a wide range and examine how this would affect nucleosome organization during replication. The other sliding parameter  $r_{s0}$  is also varied from 0.05 to 1 bp<sup>-1</sup> s<sup>-1</sup>.

**Details of simulation.** In this paper, given the events and rates, we simulate the system using kinetic Monte Carlo methods (Gillespie algorithm) (40). We start from a specified parental nucleosome profile (occupancy pattern), and simulate replication, as per the events discussed above, and produce nucleosome organization in the daughter chromatin. We repeat this many times (typically 5000) and compute average occupancy of nucleosomes on the daughter cells. Occupancy at any position  $i$  is defined as the probability that the site is covered by a nucleosome. Rates used for each figure is given in the text.

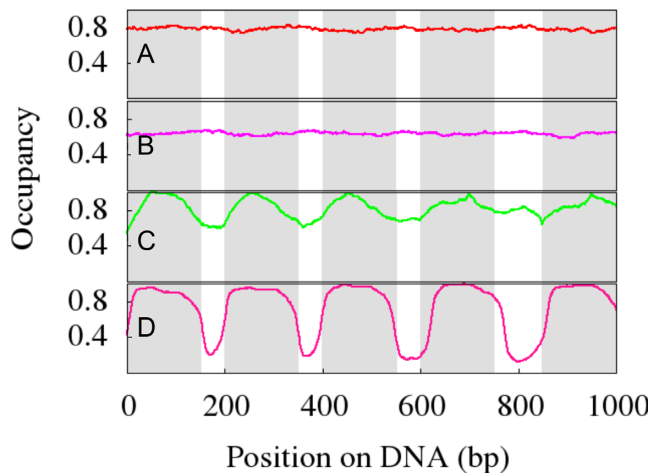
## RESULTS

### A minimal model and its limitations

The simplest (or minimal) model for replication is to consider only two processes, namely the replisome movement and the nucleosome deposition. That is, imagine a one-dimensional problem of a replication fork moving at a rate  $v_r$  and nucleosomes being deposited behind the fork with a rate  $k_{on}$ . This problem was considered by Osberg *et al.* (34). As a start, we also simulated replication with only these two processes and the results are presented in the SI text (Supplementary Figure S1). Our main findings from this simple study are (i) the average density of nucleosomes, within this minimal model, is determined by the ratio of  $v_r$  to  $k_{on}$  (ii) within this model, the density of the nucleosomes (the fraction of DNA covered by nucleosomes) has to be between 75% and 100% (iii) the occupancy pattern in this simple model will always be uniform, one will never obtain a heterogeneous (space-dependent) nucleosome organization on an average (see Figure 2A). The last two points are major limitations of the minimal model. Within this model, there is no mechanism that transfers the positional information from the parent to the daughter.

### Heterogeneous nucleosome organization : role of fork pausing and nucleosome sliding

In the simulation of the minimal model, we did not account for the experimentally observed (30) nucleosome repositioning (sliding). We also assumed that nucleosomes ahead of the replication fork get disassembled infinitely fast, resulting in unhindered (no pause) movement of the fork. However, in reality the replication might pause until the nucleosome ahead of the fork is removed. Given that nucleosome insertion behind the fork is strongly coupled with the movement of the fork (27,30), we hypothesise that the timescale of such pausing, and hence, the pausing in movement of the replication machinery, can be important in determining the nucleosome organization behind the fork. Therefore, as discussed in the model section, we introduce both sliding of nucleosomes behind the fork and pausing of the fork due to the removal of nucleosomes ahead of the fork. Each nucleosome, after deposition behind the fork, will be slid for a time  $\tau_s$  as discussed in the Model section.

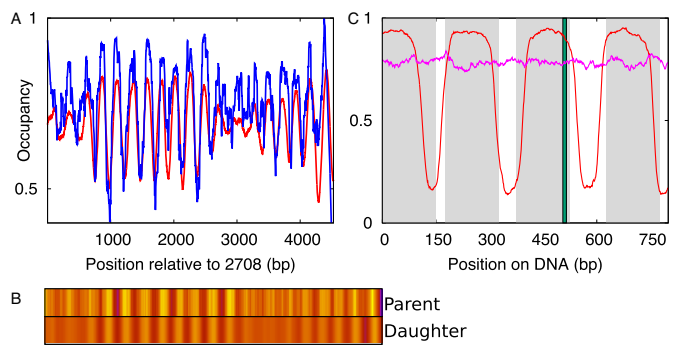


**Figure 2.** The grey regions represent nucleosome positions on the parental DNA having heterogeneous linker lengths. The resultant daughter nucleosome occupancy for various replication rules, averaged over 5000 realizations, starting from the same parent. (A) Using the minimal replication model having only two parameters:  $v_r = 500$  bp/s and  $k_{on} = 0.1$  bp<sup>-1</sup> s<sup>-1</sup>. (B) same as (A) but with nucleosome sliding added to the minimal replication model having sliding parameters  $\tau_s = 1$  s and  $r_{s0} = 1$  bp<sup>-1</sup> s<sup>-1</sup>. (C) same as (A) but with fork pausing added to the minimal model having  $\tau_p = 10$  s. (D) The full model; that is, when both pausing and sliding events are considered in addition to the fork velocity and nucleosome deposition—this replicates nucleosome positioning quite accurately. The parameter values are  $v_r = 500$  bp/s,  $k_{on} = 0.1$  bp<sup>-1</sup> s<sup>-1</sup>,  $\tau_s = 1$  s,  $\tau_p = 10$  s.

As the fork reaches a nucleosome on the parent strand, the fork will pause until a time  $\tau_p$  which is the time needed for clearing the way for the machinery to go forward by removing the nucleosome ahead. Since we do not know the precise values of these two parameters, we will vary them systematically and investigate the parameter regime under which one can observe experimentally sensible results. We, first, take the bare sliding rate as  $r_{s0} = 1.0$  bp<sup>-1</sup> s<sup>-1</sup>. The precise value of  $r_{s0}$  may not be important as we discuss later.

We start our simulation with only three moves: replisome movement, nucleosome deposition and nucleosome sliding (i.e. minimal model + nucleosome sliding; assume pausing is negligible). The results are given in Figure 2B. One can see that, with sliding and no pausing, the resulting average occupancy is homogeneous in space, and looks very different from the parental nucleosome positioning. This means that sliding cannot produce heterogeneous nucleosome positioning. Then, we simulate another limit with no sliding but with pausing (i.e. minimal model + nucleosome pausing; assume sliding is negligible). The results are in Figure 2C. Here, we find that the introduction of pausing brings some signature of the parental nucleosome organization. However, the occupancy pattern is not very similar to that of the parent.

Further, we simulate the model by introducing all the four events: fork movement, nucleosome deposition, sliding and pausing events simultaneously. First, we take the pausing timescale longer than the sliding timescale ( $\tau_p = 10$  s,  $\tau_s = 1$  s). In this parameter regime, the parental nucleosome occupancy is nicely replicated in the daughter (Figure 2D). Note that even the heterogeneity in spacing is inherited in the next generation. For example, near position 200, the gap



**Figure 3.** Comparison of nucleosome organization in parent and daughter chromatin. (A) Nucleosome occupancy in parent (blue) and daughter (red) for *S. cerevisiae* (chromosome 1: 2708–7234). (B) Same data as in (A) shown as heat map. The yellow regions represent the higher nucleosome occupancy and red regions represent lower nucleosome occupancy. (C) Nucleosome organization in PHO5 promoter region when the promoter is in ‘off’ (inactive) state. Parental nucleosomes are shown as grey bars and the TATA box (green bar) is covered with a nucleosome. Nucleosome occupancy after replication (red curve) results in covered TATA box with  $\approx 90\%$  probability. On the other hand, if we switch off pausing and sliding events, the TATA box will be covered with probability 75% (magenta curve). The parameters used to generate daughter cell nucleosome occupancy are  $v_r = 500$  bp/s,  $k_{on} = 0.1$  bp<sup>-1</sup> s<sup>-1</sup>,  $\tau_s = 5$  s,  $\tau_p = 10$  s.

between two nucleosomes in the parent is small ( $\approx 50$  bp), and near position 800, the gap is large ( $\approx 100$  bp). One can see that in the daughter cell (even after averaging over many cells) the gap variation is reproduced (Figure 2D).

In Figure 3A, we present a natural scenario where nucleosome positioning on chromosome-1 of *Saccharomyces cerevisiae* starting at location 2708 bp is replicated. We started with data obtained from Kaplan *et al.*'s study (8) (blue curve Figure 3A) as the parental nucleosome positioning profile, and performed the replication simulation on an ensemble of configurations; the resulting nucleosome occupancy of the daughter chromatin is shown as red curve in Figure 3A (also see Figure 3B). Comparing the parental and daughter nucleosome occupancy, we note the following points: the daughter occupancy is not exactly the same as the parent; however, there is a good amount of similarity where the daughter occupancy profile captures essential signatures of the parent. For example, the peak positions (high occupancy regions) are largely similar, even though the height of the peaks (and depth of the troughs) do not match well. This is qualitatively comparable to some of the recent experimental studies where there are some signatures of inheritance but the inheritance is not perfect (32).

Further, we examined the promoter region of PHO5 gene which is known to show diverse behaviour (7,41). For example, if the TATA protein binding site is covered with a nucleosome, the promoter will mostly be in the ‘off’ (inactive) state; on the other hand if TATA site is exposed, then the promoter will mostly be in the ‘on’ (active) state. Based on the recent experimental data (41), we started with a nucleosome occupancy pattern that represents the inactive (off) state of the promoter (see Figure 3C)—that is, TATA site is covered. After replication, if the nucleosome positioning is not faithfully inherited, it may lead to unwanted spurious gene expression. In our simulations, we find that with large

enough pausing, the nucleosome positioning can be inherited keeping the TATA box covered with a probability 0.9, and hence the promoter is inactive. In comparison, in the absence of pausing and sliding, the inheritance is poor—it leads to reduced coverage of TATA box (see Figure 3C). In our simulations, we rarely got configurations that are devoid of nucleosomes implying that such nucleosome free states are only possible with active remodeling (7,41).

Going beyond single genes, to understand how parameters values affect the inheritance, we have systematically studied the inheritance of nucleosome positioning by taking a few different values of  $\tau_p$  and  $\tau_s$ . In Figure 4A, we have compared nucleosome occupancies in parent and daughter chromatins for different values of  $\tau_p$  and  $\tau_s$ . We observe that whenever both  $\tau_p$  and  $\tau_s$  are non-zero, and  $\tau_p \geq \tau_s$  the daughter cell inherits the parent positioning reasonably well. To compare the nucleosome occupancies, we define deviation,  $\chi$ , as a measure of the difference in nucleosome occupancy between the daughter and the parent,

$$\chi = \sqrt{\frac{1}{L} \sum_{i=1}^L (m_i - d_i)^2}, \quad (1)$$

where  $m_i$  and  $d_i$  are occupancy of  $i$ th site in parent and daughter strand, respectively. If the nucleosome occupancy pattern between the parent and daughter is identical, then we expect the  $\chi \rightarrow 0$ ; if the occupancy patterns are very different we expect a large value of  $\chi$  close to 1. In Figure 4B, the deviation ( $\chi$ ) is plotted for different values of  $\tau_p$  and  $\tau_s$  as a heat-map with small values of  $\chi$  represented by a dark violet color and large values of  $\chi$  represented by a yellow color (see the colourbar on the side). This further verifies that for the parameter regime,  $0 < \tau_s \leq \tau_p$ , the deviation is small. That is, for  $0 < \tau_s \leq \tau_p$  the daughter somewhat faithfully inherits parental nucleosome occupancy. In SI Text (Supplementary Figure S2) we present similar results for a different set of parameter values, and it suggests that the phenomena of nucleosome positioning inheritance due to the pausing is independent of the precise parameter values we use. Please note that even for the best inheritance, the deviation is non-zero suggesting that the inheritance is not perfect. However, the process lays down a pattern of nucleosome positioning similar to the parent and this may help the post-replication maturing events in achieving a proper steady-state nucleosome organization.

### Role of strongly positioned nucleosomes and barrier-like proteins

In certain parts of chromatin, it is known that there are regions where nucleosomes are ‘strongly’ positioned, while other regions have weakly positioned nucleosomes (8,42,43). Even though the DNA sequence may influence the regions with strong positioning, it is well known that factors beyond the sequence also affect nucleosome stability. For example, action (or the lack of action) of certain remodellers, histone variants (H2A.Z, H3.3), various nucleosome-binding proteins (like H1 or HMG family proteins) and histone modifications are all known to affect the stability and positioning strength of nucleosomes (44–49). Does stability/positioning-strength of

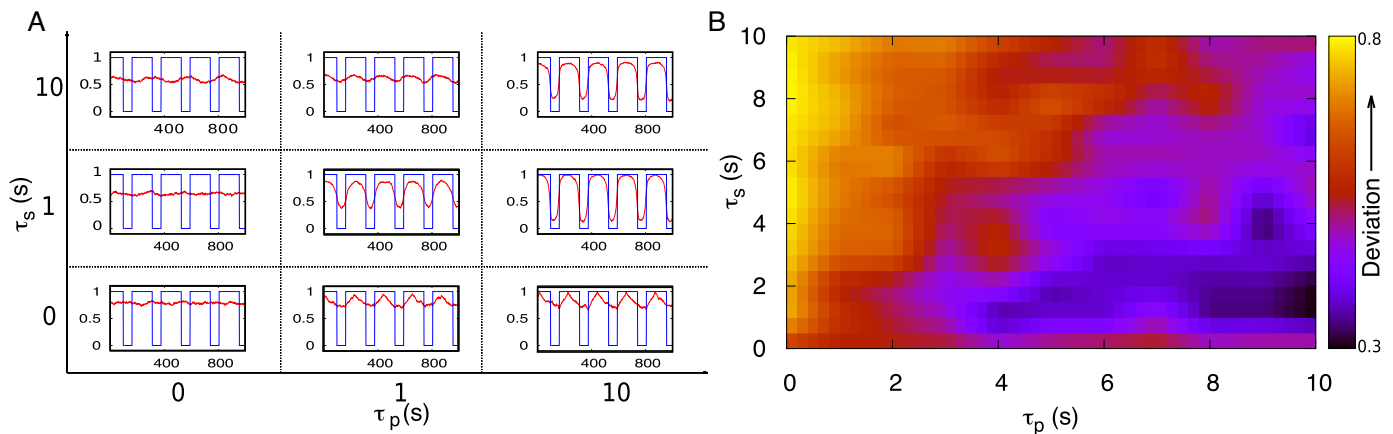
nucleosomes have any role in transferring the nucleosome positioning information into the daughter cells?

We investigate the effect of strong vs weak nucleosome positioning and how they influence the occupancy pattern in daughter chromatin. Strongly positioned nucleosomes are defined as those nucleosomes that are more difficult to be disassembled ahead of the fork – that is, nucleosomes having a higher value of  $\tau_p$  are strongly positioned, while low  $\tau_p$  would imply weakly positioned nucleosomes. We simulate such a system with heterogeneous (high and low)  $\tau_p$  values 0.01 s (weak) and 10 s (strong) keeping  $\tau_s$  (=1 s) fixed. In a long stretch of DNA, we consider two special regions with strongly positioned nucleosomes. In Figure 5A, the two grey-shaded regions (each of length 365 bp) contain two strongly positioned nucleosomes each, while the rest of the DNA has weakly positioned nucleosomes. All nucleosomes are arranged with a uniform linker length of 65bp. The resulting nucleosome positioning in the daughter cells (averaged over 5000 cells) is shown as a red curve. We observe that strongly positioned parental nucleosomes give rise to regions in daughter chromatin with high nucleosome occupancy inheriting the strong positioning. Also note that there is a statistical positioning on either side of the strongly positioned nucleosomes implying that the strongly positioned nucleosomes can influence the positioning of the neighboring nucleosomes like in the case of the well-known statistical positioning near a strong ‘barrier’ (9,14,15). In SI text (Supplementary Figure S3), we show that a similar inheritance of nucleosome positioning is applicable even when just one nucleosome is strongly positioned (also see Supplementary Figure S4).

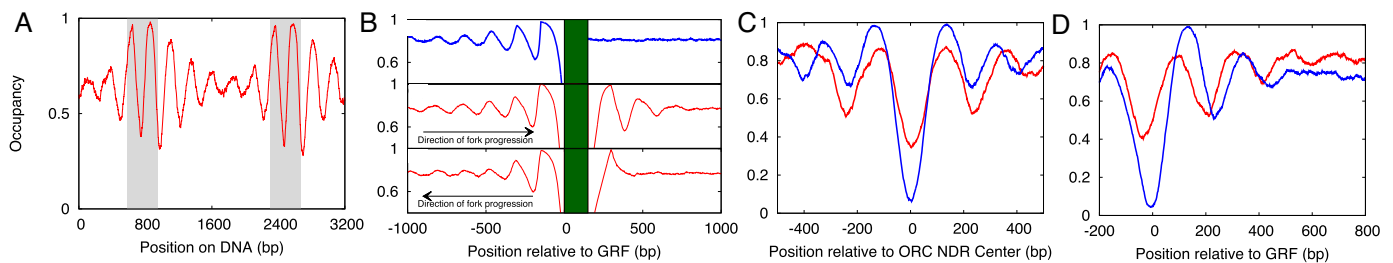
Another aspect of such local nucleosome positioning influenced by various proteins happens in the context of gene-regulatory factors (GRF). We consider a situation where there is certain non-histone GRF present in the parental gene. It is known that when a bound GRF is highly stable, it can act like a ‘barrier’ and cause statistical positioning (9,14,15,50) of nucleosomes. Typically, it is known that the coding region will have the statistical positioning of nucleosomes, while the regions upstream to TSS often show different kinds of nucleosome organization (9). How the nucleosome positioning is inherited near a GRF is an interesting question, and recent works have probed this experimentally (22,30,32). Here, we examine the prediction of our model given certain nucleosome organization reminiscent of GRF locations on the parent DNA.

On the parent DNA, on the left side of the GRF we start with the statistical positioning of nucleosomes, and on the right side with uniformly positioned nucleosomes (flat occupancy) with mean density  $\approx 85\%$  (see top panel of Figure 5B). We start with 5000 parent copies of the same gene, each having nucleosomes organized near the GRF in such a way that the mean of the occupancy of the parents as given in the top panel of Figure 5B. Each of these 5000 copy is replicated once, and we look at the nucleosome positioning on each of the gene and compute the average occupancy, which is plotted as red continuous curve in Figure 5B.

When we carry out the replication from left to right with regard to the GRF (in the parent, the left side has statistical positioning, the right side has uniform occupancy), we find that on the left side the statistical positioning gets



**Figure 4.** Comparison of nucleosome organization between parent and daughter chromatin for various pairs of  $(\tau_p, \tau_s)$  values. **(A)** Blue sharp curves represent the parental nucleosome organization and the red curves represent daughter nucleosome organization averaged over an ensemble of realizations. **(B)** Heat-map for the quantity ‘deviation’ ( $\chi$ ) as defined in Eq. (1),  $\chi$  increases as color varies from violet to yellow. For  $0 < \tau_s \leq \tau_p$  there is less deviation from parent to daughter nucleosomal organization. The parameters used to generate daughter cell nucleosome occupancy are  $v_r = 500$  bp/s,  $k_{on} = 0.1$  bp<sup>-1</sup> s<sup>-1</sup>,  $r_{s0} = 1$  bp<sup>-1</sup> s<sup>-1</sup>. For a different parameter value of  $r_{s0} = 0.05$  bp<sup>-1</sup> s<sup>-1</sup>, the results are shown in SI Supplementary Figure S2.



**Figure 5.** **(A)** The simulations are performed with parental nucleosomes in the grey shaded region (two nucleosomes in each grey region) that are strongly positioned ( $\tau_p = 10$  s) and other nucleosomes that are weakly positioned ( $\tau_p = 0.01$  s) with uniform linker length of 65 bp. The daughter nucleosomal organization (occupancy) for such a heterogeneous fork pausing times is shown as the red curve. Other parameters are kept constant as mentioned below. **(B)** Nucleosomal positioning information transfer in the vicinity of gene regulatory factors (GRF). Top panel blue curve represents the parental nucleosome organization and the green solid bar shows presence of GRF. The middle panel shows nucleosome positioning in the daughter chromatin when the replication is performed from left to right and the bottom panel shows nucleosome positioning in the daughter chromatin when the replication is performed from right to left. We have also performed similar simulation for symmetric nucleosome organization on either sides of GRF on the parent gene (see SI text figure Supplementary Figure S5 (A)) with asymmetric parameters on either side of the barrier (Supplementary Figure S5(B)). **(C)** Nucleosome occupancy reflecting competition between nucleosomes and non-histone proteins near origin recognition complex(ORC) binding site. The parental curve (blue, in steady state) differs from the daughter curve (red, immediately after replication). The difference arises because nucleosomes compete with non-histone proteins binding at nucleosome depleted region (NDR) which represents ORC binding site. **(D)** Similar scenario as in (C) but with GRF-nucleosome competition near transcription start site (TSS). Here too the parental curve (blue, in steady state) differs from the daughter curve (red, immediately after replication) because nucleosomes compete with GRFs. In (C) and (D), nucleosomes and non-histone proteins bind at NDR with equal probability. Unless specified otherwise, in all the four graphs (A–D) the common parameters used are:  $v_r = 500$  bp/s,  $k_{on} = 0.1$  bp<sup>-1</sup> s<sup>-1</sup>,  $\tau_p = 10$  s,  $r_{s0} = 1$  bp<sup>-1</sup> s<sup>-1</sup>,  $\tau_s = 1$  s (A, B) or 5 s (C, D).

replicated fairly well (see middle panel of Figure 5B). However, on the right side, even though there was a flat positioning in the parent, the daughter chromatin has nucleosomes with non-uniform oscillatory occupancy in space. The physical reason for this is the following: on the left side, daughter gene inherits the parental occupancy via pausing and sliding; Whereas, on the right side, due to the effect of the GRF barrier, one obtains oscillatory positioning—it is well known that nucleosomes near a barrier will have spatial oscillations in occupancy. This also indicates that physical barriers will have influence near the barrier site, even with pausing and sliding. In our simulations, since the GRF is bound immediately behind the replication fork, the nucleosome depositing after the GRF ‘feels’ (via steric exclusion) the GRF barrier, and hence, the generation of the oscillatory pattern. Please note that ATPase activity (here, sliding

of nucleosomes) is an important factor in producing the oscillatory pattern as known in other contexts (9,14).

When we carry out the replication from right to left with respect to GRF, we get the result as shown in Figure 5B, bottom panel. Since the machinery that is moving towards GRF is unaware of the presence of GRF until it reaches the location, the replicated chromatin will have very little influence of the barrier. However, after the GRF, the statistical positioning is reproduced. Within the short span of sliding, the nucleosome very close to the GRF feels the barrier and hence, one obtains a single peak on the right side (Figure 5B, bottom panel). We observe that the nucleosome organization immediately after the replication in the vicinity of GRF is tied to the replication fork progression direction (see Figure 5B). This positioning may change long after replication under the influence of other events such as transcription or

action of various remodellers (32). These local remodelling events may destroy the spontaneous peak formed in Figure 5B and lead to parent-like nucleosome positioning as a result of these extra events.

So far we have assumed that non-histone proteins like GRFs bind in the nucleosome depleted region (NDR) with large affinity and occupy their precise locations on the DNA. However, many of the recent experiments indicate that nucleosomes compete with non-histone protein binding and this may result in gain of nucleosomes in NDR region (22). To test this, we introduced the competition between nucleosome binding and binding of non-histone proteins (binding factors near replication origin and GRFs near promoters) in the following way. Whenever a non-histone protein binding region is replicated, that newly replicated region is free to be occupied by nucleosome and non-histone protein with probabilities  $(1 - \alpha)$  and  $(\alpha)$ , respectively. Typical transcription factor binding free energy ( $\approx 5-15$  kcal/mol) can be comparable to the nucleosome binding free energy at certain sequences (14,51,52). Experiments have also shown that, at biologically relevant concentrations, typical transcription factors can have binding rates comparable to that of nucleosomes (51). Hence we consider  $\alpha = 0.5$  here. In Figure 5C, we present our results of nucleosome positioning near origin of replication and find that the inheritance is poor when the non-histone protein binding probability is small. This is similar to the experimental observations made by Ramachandran and Heinikoff in their recent paper (22). We also find that the competition mostly leads to nucleosome gain in the nucleosome depleted regions and it influences the inheritance. A similar picture is also obtained at promoter region where a GRF is competing with nucleosomes (see Figure 5D). This suggests that the inheritance of nucleosome positioning also depends on other factors such as action of non-histone proteins. Therefore, in some context, transcription may also play an important role in ‘maturation’ of nucleosome positioning as indicated in (32).

## DISCUSSION

In this paper, we have addressed the question of inheritance of nucleosome organization from parent to daughter, instantly after replication, by simulating a plausible physical model. We have used various known information from published experiments and constructed a model to study the effect of different replication-related processes on nucleosome organization in daughter cells. We have first studied a bare minimum model of the fork movement and nucleosome deposition behind the fork, which can only produce a homogeneous nucleosome distribution in the daughter cell irrespective of parental organization. Since the bare minimum model has no mechanism to transfer information of the heterogeneous parental nucleosome organization to the next generation, we have introduced another physically important process, which is the pausing of the replication fork on encountering a nucleosome on the parental chromatin. This interaction of the fork with nucleosomes have given some signature of parental organization in the daughter strand, but the signature has not been precise enough. Consequently, we introduced sliding of the newly deposited nu-

cleosomes as reported in recent experiments (53,54). Using computer simulation we explore the parameter-space and show that when one has a finite pausing and sliding with comparable timescales, one gets replicated daughter chromatin that has similar nucleosome occupancy as that of the parental chromatin. Our model argues that strongly positioned nucleosomes act as ‘barriers’ that will make the replication fork pause for a short period (a period comparable to the nucleosome sliding timescale) at the site of the strongly positioned nucleosomes, and this pause will help transferring the positioning information from parent to daughter.

## Nucleosome positioning inheritance at ‘inactive’ gene regions

In the first part of our paper, we have only accounted for events that would typically occur in an ‘inactive’ gene region, namely, nucleosome disassembly and related pausing ahead of the fork, DNA replication, nucleosome deposition behind the fork and nucleosome sliding. With these events, we find that the nucleosome positioning can be inherited given that the pausing timescale is sufficiently big ( $\tau_p \geq \tau_s$ ). As discussed in the context of Figure 3, a randomly selected typical gene region with no extra activity due to non-histone proteins, and an inactive (off) gene promoter region (e.g. PHO5) can inherit nucleosome positioning from the parental chromatin. This is consistent with recent experimental observation that ‘nucleosome positions are conserved at inactive sites behind replication fork’ (22).

Our results do not imply that, with pausing, the inheritance is perfect. There is always some finite amount of deviation (e.g. Figures 3A and 5B). What our work suggests is that if pausing happens, it allows the chromatin to pass some information about the nucleosome positioning to the daughter chromatin. The duration of pausing will crucially depend on the local nucleosome stability and hence it may be highly heterogeneous. As our results show, if the nucleosomes are not stable, the pausing and inheritance will be negligible. Interestingly, nearly all the experiments that study nucleosome positioning behind the fork report non-homogeneous (having peaks and troughs) nucleosome occupancy pattern immediately after replication (22,32). As we show in our work, a minimal model would not give rise to this heterogeneity (Figure 2). Our results suggest that nucleosome pausing would lead to inheritance of the inhomogeneity seen in the parental chromatin. Therefore, one of the interesting predictions of our model is that pausing would play a role in giving rise to heterogeneity in nucleosome organization.

## Lack of nucleosome positioning inheritance at ‘active’ gene regions

At ‘active’ gene regions, non-histone proteins play important role—for example, gene regulatory factors. In the second part of the paper, we extended our model incorporating binding of different non-histone proteins that compete with nucleosomes to occupy certain specific sites along the genome. Our results show that this competition will lead to imperfect inheritance of nucleosome positioning (Figure 5C and D). This is also consistent with (22) where they find a lack of inheritance in nucleosome positioning at such active sites. This again suggests that many other factors could

influence the positioning of nucleosomes in the daughter chromatin. Depending on the gene location and the factors involved the precise nature of nucleosome positioning inheritance may vary.

### Strength and limitations of the model

The strength of our model is that it incorporates various known experimental features such as nucleosome deposition behind the replication fork, sliding of newly deposited nucleosomes, and physically plausible events like nucleosome pausing. In our work we do not distinguish between replication of leading versus lagging strand. We find that if fork pauses for sufficiently long time and there is sufficient time for remodelling machines to position nucleosomes, the results that we obtain should be similar for both the strands. Since the mechanism of replication is different for the lagging strand, we tried to mimic that in a modified simulation relevant for the lagging strand (see SI text section 5 and Supplementary Figure S6). We find that results do not change significantly. However, the model has various limitations or drawbacks: the first drawback is that we have not considered the extended size of replisome, which is  $\approx 55$  bp long (55). One reason we did not put in the size of a replisome is that, during the pause, it may happen that the replisome would partially unwrap or partially disassemble the nucleosome (which can be of a few tens of basepairs comparable to the size of the replisome) before pausing close to the dyad; this will offset the effect due to the finite size of the replisome and we will end up with a scenario that is very similar to what we have obtained here. In other words, we have not considered the size of a replisome, while we have assumed that the nucleosome at the fork will occupy full 150 bp; however, the reality might be that the nucleosome may unwrap occupying only  $<100$  bp, while the rest of the space might be occupied by the replisome. In the case of transcription, it has been reported that the RNA Polymerase pauses inside the unwrapped nucleosome near the dyad region (38). This would be mathematically equivalent of what we did and it will not change our results. The second limitation is that we have considered nucleosomes as stable hard-core particles—that is, particles with strong steric repulsion disfavoured any amount of overlap. However, partial unwrapping of DNA from nucleosomes has been observed experimentally (56); this feature as discussed in earlier works (34,57,58) is not included in the current model and may be addressed in a future work. Another limitation is that the rates of processes *in vivo* might be very different from what we have taken for our simulations. However, we have explored the parameter-space, and found that our results will not depend on the precise value of rates; rather, the results will be true for a range of rates.

### Suggestions for new experiments to test our predictions

Our work predicts that strongly positioned nucleosomes will induce a pause in the progression of the replication fork, and this pause will help in transferring nucleosome positioning information from parent to daughter. One way to test our predictions is to do experiments with and without strongly positioned nucleosomes in the parent chromatin.

One possibility would be to make appropriate modifications to histones that would stabilise/destabilise the nucleosomes. This may be achieved by using appropriate histone variants or by using suitable chemical modifications along the histone tails. It can be tested whether a less stable (more stable) nucleosome positioning is poorly (better) inherited or not. Another way would be to stabilise nucleosomes on the parent chromatin by inserting artificial sequences (like the 601 sequence). Since the sliding machinery is known to slide nucleosomes away even from 601-like strongly positioning sequences (18), the pause will contribute to the inheritance of nucleosome positioning at such strongly positioned locations.

### CONCLUSIONS

Our study can be a first step in the direction of understanding the mechanism of inheritance of epigenetic information from parent to daughter, and it introduces strong physical arguments with predictive power. With our model we have been able to reproduce the parental nucleosome organization in the daughter cell with reasonable precision after disruption due to replication. While in some regions, the remodeling after replication (e.g. nucleosome rearrangement related to transcription (32)) might play important role, for some other regions (like heterochromatin or regions where the gene is ‘off’) the positioning of nucleosomes after replication may not change much. Hence, the inheritance of precise nucleosome positioning in these regions can be crucial; an erroneous gene activation due to incorrect epigenetic information transfer during replication may lead to various abnormalities and diseases (59,60). Our results will certainly be important for these latter regions. Even for regions that may change their nucleosome positioning after transcription, it is important to have a proper nucleosome positioning at all times as incorrect nucleosome positioning may expose promoters leading to unwanted transcription. We know that there are many other factors, such as DNA sequence and chemical modifications of histones, which also play significant roles in deciding the nucleosome organization. Further study is required to quantify the significance of these factors at various stages of the cell cycle.

### SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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