Brownian dynamics simulation of the cross-talking effect among modified histones on conformations of nucleosomes*

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Using Brownian dynamics simulation, we studied the effect of histone modifications on conformations of an array of nucleosomes in a segment of chromatin. The simulation demonstrated that the segment of chromatin shows the dynamic behaviour that its conformation can switch between a state with nearly all of the histones being wrapped by DNA and a state with nearly all of the histones being unwrapped by DNA, thus involving the "cross-talking" interactions among the histones. Each state can stay for a sufficiently long time. These conformational states are essential for gene expression or gene silence. The simulation also shows that these conformational states can be inherited by the daughter DNAs during DNA replication, giving a theoretical explanation of the epigenetic phenomenon.

Keywords: nucleosome, histone modification, DNA, Brownian dynamics

PACC: 8715, 8715B, 8220W

1. Introduction

Histone and DNA are the basic components of the nucleosome in a eukaryote cell and much of the crystal structure of the nucleosome is known. In one nucleosome, about 146-bp DNA is wrapped around a histone octamer which contains pairs of each of the histone proteins, H2A, H2B, H3, and H4.[1−3] Each of them also contains unstructured N-terminal tail domains ranging from 15 to 35 amino acids, which are extended out of the histone particle.[4] Histone tails provide sites for various post-translational modifications, including acetylation, methylation, phosphorylation and ubiquitination of lysine (K) residues, phosphorylation of serine (S) and threonine (T) residues, and methylation of arginine (R) residues.[5] It is believed that such modifications of histone tails determine the interaction of histone with other proteins which may also regulate the structure of nucleosomes.[6] In general, the acetylation of histone is usually associated with the activity of transcription while deactelytion is often associated with inactivity. The effect of methylation and demethylation is more complicated: methylation can either activate or depress transcription.[7] It is generally believed that the modification changes are often associated with stable changes in chromatin structure and subsequently influence the transcriptional activation of the DNA region.[8] Some evidence also shows that the modification state of a histone can affect the modification state of another histone and these effects are often not only restricted to the single histone but also to an array of histones in chromatin.[9−13]

It has been found that the modification at specific positions in histone tails involves a class of enzymes, including histone acetyltransferases (HATs), histone methyltransferases (HMTs), histone deacetylases (HDACs) and histone demethylases (HDMs[14]). Some of the modifications caused by these enzymes influence the activity of nearby genes, for the reason that the modifications can affect the binding of regulatory proteins to nucleosome. If modified histone recruits some of these enzymes which can produce the modification state of the histone itself and interact with other histones by changing their modification states, a positive feedback is formed. Thus, an array of nucleosomes can then maintain itself stably in a particular modification state. The modification pattern is proposed to be able to be inherited by the daugh-

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ter DNA strands during replication. Upon replication, the parental nucleosomes are believed to be partitioned randomly between the two daughter strands, and new nucleosomes are inserted or assembled to fill the gaps.[15] Enzymes recruited by these nucleosomes can then establish the modification pattern of the new deposited nucleosomes as their parental DNA. That is called "epigenetic" memory.

Researchers have theoretically analysed the positive feedback interaction of histone modification and obtained bistability of the modification state of the chromatin.[16] Some experimental evidence also shows bistability.[17,18] An approximately 20-kb region of chromosome II of *Schizosaccharomyces pombe* that contains two mating-type cassettes, mat2-P and mat3-M, is normally in a stable silenced state, in which the mating-type genes are not expressed. A portion of this region is removed and a report gene ura4$^+$ as mutant is then inserted. It is found that the expression of the report gene is bistable, switching between silenced state where ura4$^+$ is repressed and active state where ura4$^+$ is expressed. Each state is remarkably stable and inheritable.[18,19] Although the theoretical analysis had been done, where a model has been built up to determine the statistical property of the histone modification state, the work does not involve the dynamics on the switch of nucleosomal conformations. The purpose of this work is to study the dynamics on the switch of nucleosomal conformations due to the effect of histone modifications.

2. Models

2.1. Stochastic model

Here we consider a DNA region containing 60 nucleosomes. The length of this region is about 12 kb, equivalent to 200 bp per nucleosome. For simplicity, we adopt a similar model to that used in Ref. [16], which is briefly stated as follows.

(i) Only three kinds of nucleosomes are considered: unmodified (U), acetylated (A), and methylated (M). The A state corresponds to the active state, while the U and M states correspond to the silenced state.

(ii) The nucleosome is actively interconverted by modifying and demodifying enzymes (HMTs, HATs, HDMs, and HDAs) that are recruited by the modified nucleosome.

(iii) The nucleosome can also be modified and demodified by the enzymes that are free in solution or attached to the nucleosomes beyond the region. This can be considered as a "noise" in our system. We use a parameter P to describe the rate of recruit effect, as stated in 2, and $1-P$ to describe the rate of "noise" effect.

(iv) The rates of the interconversion reactions at each nucleosome across the region are the same, independent of the nucleosome position.

Moreover, the model is carried out by the following steps in one time unit.

Step 1 A random histone n1 to be modified is selected in a DNA region that contains $N = 60$ nucleosomes. With a probability P, the system goes along step 2A, or, with a probability $1-P$, it goes along step 2B.

Step 2A Another random histone n2 is selected within the region. i) If n2 is in either the A or the M state, the state of n1 will be changed by one step toward that of n2. That is, if n1 is in the A (M) state and n2 in the M (A) state, n1 will be changed to the U state; if n1 is in the U state and n2 in the M (A) state, n1 will be changed to the M (A) state. ii) If n2 is in the U state, the state of n1 will be kept unchanged. This process can be considered as a positive feedback.

Step 2B If n1 is in the A state, it will be changed by one step toward the M state, with a probability of 1/3. If n1 is in the M state, it will be changed by one step toward the A state, with a probability of 1/3. If n1 is in the U state, it will be changed by one step toward the A or the M state, with a probability of 1/3. This process can be considered as noisy conversion.

Note that, in Step 2, no direct conversion between the A and M states can occur. The purpose of one-third probability in Step 2B is to make the rate of recruit conversion and the noise conversion equal when $P = 1-P = 0.5$ and the numbers of each type of histones are equal: $N_{\rm A} = N_{\rm M} = N_{\rm U}$.

It is useful to define a feed-back-to-noise ratio $F = P/(1-P)$. As will be seen in the following section, the time t can be fitted in our dynamic simulation.

2.2. Brownian dynamics model

We use the Brownian dynamics model[20,21] to describe motion of DNA and histones. In this model, DNA chain is modeled as a semiflexible homopolymer chain which consists of N spheres connected by bonds. As the width of the DNA molecule is about 2 nm, we take the diameter of each sphere to be 6 bp.

The histone octamer is also modeled as a bead, with a diameter of 5.6 nm, which is consistent with that determined from the crystal structure.

The self-avoiding potential of the DNA molecule is described by using the repulsive part of Morse potential

$$U_{m,\mathrm{rep}} = \varepsilon_m k_\mathrm{B} T \sum \exp[-\alpha_m (r_{i,j} - \sigma_m)], \qquad (1)$$

where ε_m represents the exclusive intensity of two DNA beads, α_m represents the width of the potential field, $r_{i,j}$ is the distance between the i-th and j-th spheres, and σ_m is the width of DNA, which represents also the equilibrium distance between neighbouring spheres. In our calculation, we take $\varepsilon_m = 0.2$, $\alpha_m = 2.4$ and σ_m as the unit length. The Boltzmann constant k_B is set to be unity and $T = 300$ K. The bond potential of the DNA chain is considered as a harmonic potential

$$U_\mathrm{bond} = \frac{k k_\mathrm{B} T}{2\sigma_m^2} \sum (|\boldsymbol{r}_i - \boldsymbol{r}_{i+1}| - \sigma_m)^2, \qquad (2)$$

where $k = 400$, $\boldsymbol{r}_i$ and $\boldsymbol{r}_{i+1}$ are the i-th and $(i+1)$-th DNA bead location vectors. We use a bending potential to describe the stiffness of the DNA chain

$$U_\mathrm{bend} = \kappa k_\mathrm{B} T \sum \left(1 - \frac{(\boldsymbol{r}_{i-1} - \boldsymbol{r}_i) \cdot (\boldsymbol{r}_i - \boldsymbol{r}_{i+1})}{\sigma_m^2}\right), \qquad (3)$$

where $\kappa = 15$.

The interaction between a histone octamer and DNA chain is described by the Morse potential

$$U_\mathrm{I} = \varepsilon k_\mathrm{B} T \sum \{\exp[-2\alpha(r_i - \sigma)] - 2\exp[-\alpha(r_i - \sigma)]\}, \qquad (4)$$

where ε represents interaction strength between the histone and DNA, $\alpha = 6.0$ and $\sigma = 1.9\sigma_m$. The diameter of the histone octamer is $2.9\sigma_m$, which is consistent with the structure. The r_i is the distance between the i-th DNA sphere and the histone octamer.

The repulsion between histones is described by using the repulsive part of the Morse potential

$$U_{\mathrm{M,rep}} = \varepsilon_\mathrm{M} k_\mathrm{B} T \sum -\alpha_\mathrm{M}(R_{i,j} - \sigma_\mathrm{M}), \qquad (5)$$

where $\varepsilon_\mathrm{M} = 0.2$, $\alpha_\mathrm{M} = 24$, $R_{i,j}$ is the distance between the i-th and j-th histones and σ_M is their equilibrium distance that is taken to be $13\sigma_m$.

The motion of each DNA sphere and the histone octamers are described by the following Langevin equations

$$\gamma_\mathrm{M} \frac{\mathrm{d}\boldsymbol{R}_i}{\mathrm{d}t} = -\nabla_i U + \boldsymbol{F}_{r,i}(t), \qquad (6a)$$

$$\gamma_\mathrm{m} \frac{\mathrm{d}\boldsymbol{r}_j}{\mathrm{d}t} = -\nabla_j U + \boldsymbol{f}_{r,j}(t), \qquad (6b)$$

where γ_M and γ_m are drag coefficients acting on each histome octamer and DNA sphere, respectively, both of which are calculated according to the Stokes law. $\boldsymbol{F}_{r,i}$ and $\boldsymbol{f}_{r,j}$ are random forces acting on each histone octamer and DNA sphere, respectively, which obey the fluctuation–dissipation theorem. U is the total potential, which is written as

$$U = U_\mathrm{m,rep} + U_\mathrm{M,rep} + U_\mathrm{I} + U_\mathrm{bond} + U_\mathrm{bend}. \qquad (7)$$

Equations (6a) and 6(b) are solved numerically by using the stochastic Runge–Kutta method. The step size is taken to be $\Delta t = 0.0003\tau$, where $\tau = \gamma_m \sigma_m^2 / 2T$ is as a unit time. We have checked that, by further reducing Δt, the final results are not changed. The interaction strength ε is the critical parameter to describe the interaction between histone and DNA spheres. As determined before, when ε is taken to be 5.0∼6.5, a DNA chain can be stably wrapped 1.5 to 2 turns around a histone, while when $\varepsilon < 4.5$, the DNA chain is unable to wrap around the histone.[21] In the present work, the value of ε is dependent on the modification state of the histone octamer: for simplicity, we take $\varepsilon = 4.0$ when a histone octamer is in the A state, while $\varepsilon = 6.0$ when the histone octamer is in either the U or the M state.

3. Results

First, we use the stochastic model to determine the parameters that can give the switching frequency between the two bistable states, the "high-acetylation" or "high-A" state and the "high-methylation" or "high-M" state, which is consistent with the available experimental data. The "high-A" state is defined as that most of the histones in the region are acetylation or the number of acetylated histones is at least one and a half more than the number of methylated histones, while the "high-M" state is as that the number of methylated histones is at least one and a half more than the number of acetylated histones. As shown in Fig. 1, two parameters, the total number of histones N and the "signal-noise proportion" F, are critical to the switching frequency between the two bistable states. The left column of Fig. 1 shows the number of histones that have the modification state A as a function of time for different values of N but with fixed $F = 2.3$. The unit of time t is defined as the average attempted nucleosome

conversion per nucleosome. From the left column of Fig. 1, it is seen that the switching frequency decreases with the increase of N. When $N < 40$, the switching frequency is too large to be consistent with the experimental data. For $N = 60$, the switching frequency is consistent with the experimental data and the further increase of N has a nearly negligible effect on the switching frequency. Thus, in this work we take $N = 60$ in the Brownian dynamics simulation. From the right column of Fig. 1, which shows the number of histones that have the modification state A as a function of time for different values of F but with fixed $N = 60$, it is seen that the switching frequency decreases with the increase of F. When $F \leq 2$, the switching frequency is so large while when $F \geq 2.7$ the switching frequency is so small that it is not consistent with the experimental data. $F = 2.3$ gives the switching frequency that is consistent with the experimental data. Thus, in our Brownian dynamics simulation we take $F = 2.3$. These values of $N = 60$ and $F = 2.3$ are also consistent with those used in Ref. [16].

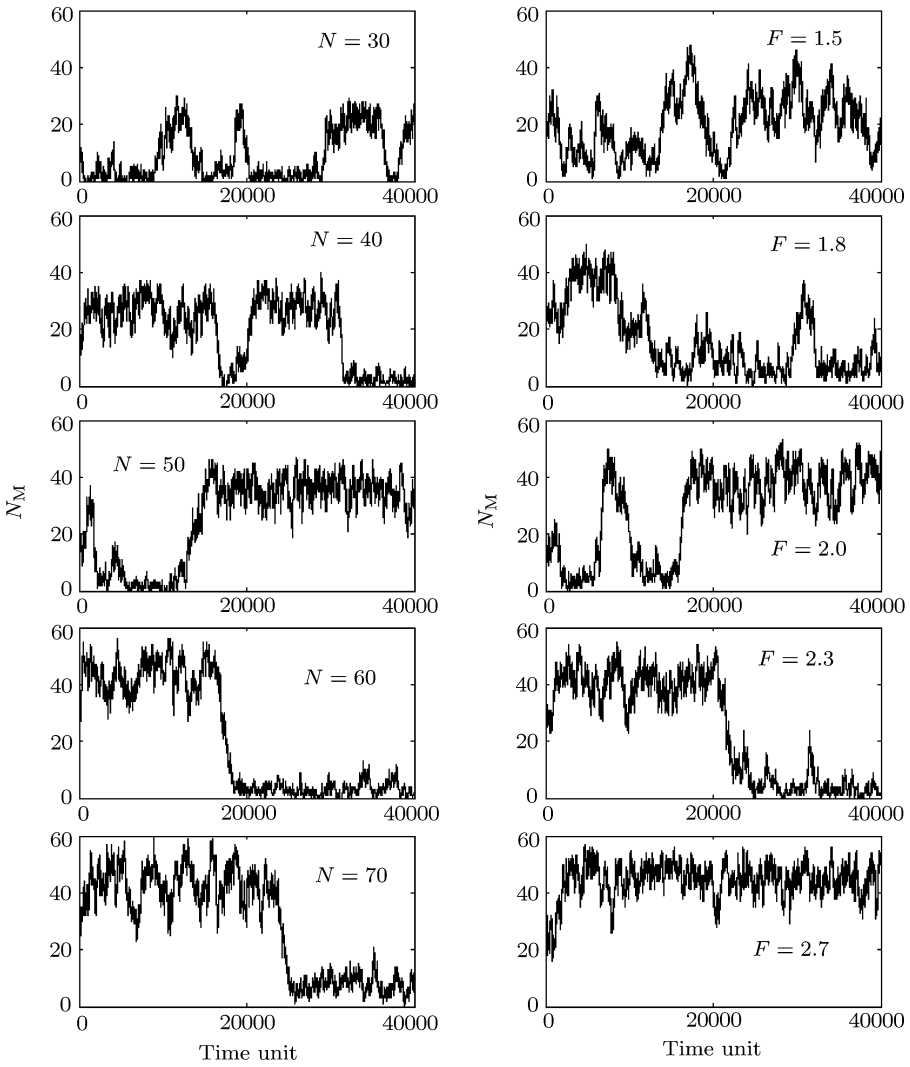


Fig. 1. The left column shows how the total number of histones, N, in a segment of chromatin affect the bistability of the modification state of the histones with $F = 2.3$. N is the total number of histones and $N_{\rm M}$ represents the number of histones that have the modification state A. As N grows, stable bistability gradually emerges. The right column shows the effect of F with $N = 60$. Stable bistability occurs when $F = 2.3$.

Then, we use the Brownian dynamics model to study the dynamics of a segment of chromatin that consists of an 11 kb length of DNA and $N = 60$ histones. For the initial ($t = 0$) condition, we randomly set the modification state of each histone at an equal probability, implying that there are about 20 histones in the U state, 20 histones in the A state and 20 histones in the M state. The corresponding conformation of the

chromatin is that about 40 histones (20 histones in the U state plus 20 histones in the M state) are wrapped with DNA (in the wrapped conformations) and 20 histones in the A state are unwrapped with DNA (in the unwrapped conformations).

In Figs. 2 and 3 we show two typical results. Figure 2 represents the case that the conformation of the chromatin switches once. From Figs. 2(a) and 2(b), we can see that the chromatin has two conformational states, "all-wrapped" state (Fig. 2(a)) and "all-unwrapped" state (Fig. 2(b)). To quantitatively describe the number of histones, $N_{\rm W}$, in the wrapped conformations as a function of t, we use a simple way to identify whether a histone is in the wrapped or in the unwrapped conformation.[21] We count the number of DNA beads which have a distance shorter than $2.8\sigma_m$ to the centre of the histone. If one histone has more than 16 DNA beads within this distance, the histone is considered to be in the wrapped conformation. Figure 2(c) shows the number of histones in the wrapped conformations as a function of time t. We can see that, for $t \leq 18000$, nearly all of the histones are in the wrapped conformations and, thus, the corresponding chromatin is considered to be in the "all-wrapped" state. After the time $t = 20000$, most histones are in the unwrapped conformations and, thus, the corresponding chromatin is considered to be in the "all-unwrapped" state until $t = 40000$.

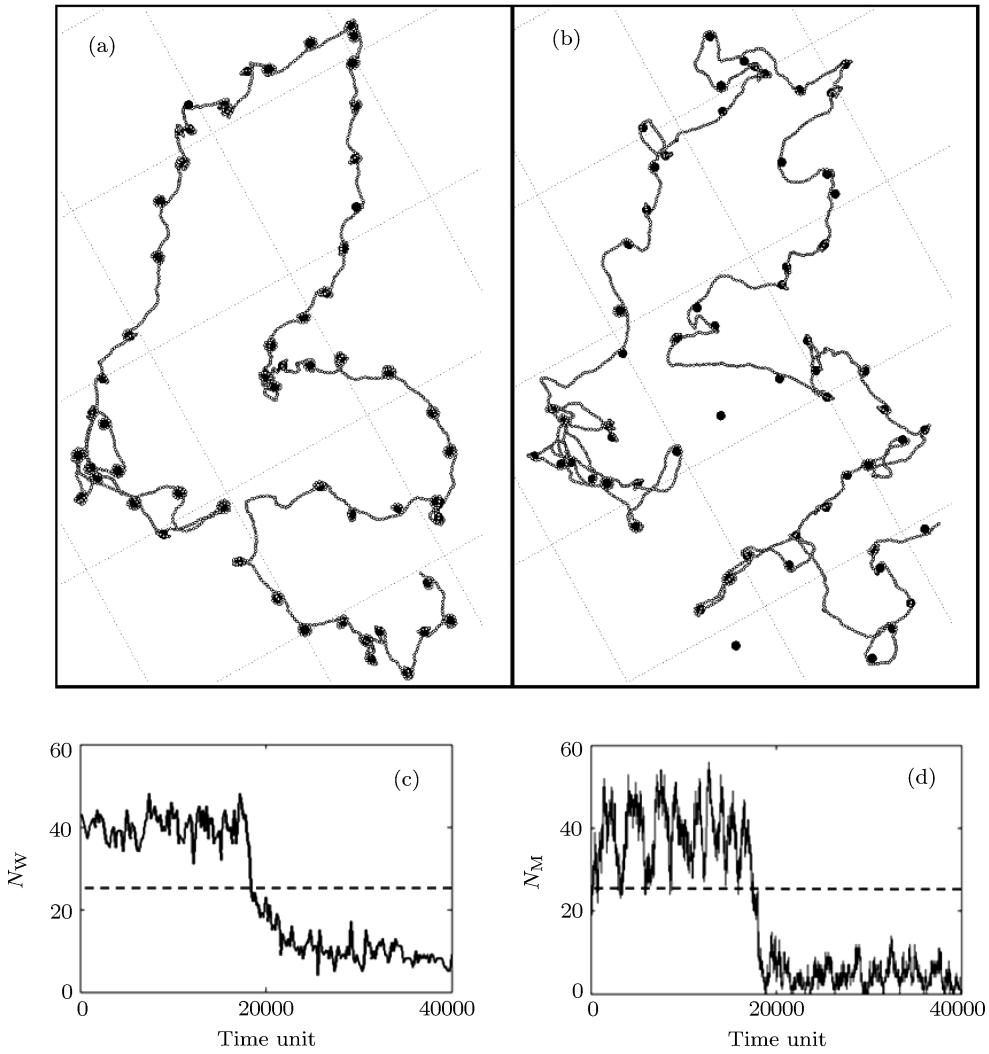


Fig. 2. (a) "All-wrapped" conformation: Nearly all histones are wrapped with the DNA chain. (b) "All-unwrapped" conformation: Most of the histones are unwrapped. (c) Statistical result by using the Brownian dynamics calculation. The $N_{\rm W}$ is the number of histones that are wrapped by the DNA chain. (d) Statistical result by using a stochastic calculation. The $N_{\rm M}$ is the number of histones which have the modification M state. The area above the dash line in (c) and (d) indicates that the histone array is in the "all-wrapped" state or the "high-M" state while under the dash line means the "all-unwrapped" state or "high-A" state.

This is in agreement with the fact that, when a fraction of a gene in the cell of an organism expresses,

the nucleosomes in that region are in the stably unwrapped conformations for a sufficiently long time. The results in Fig. 2(c) are also consistent with the prior experimental results, showed that histone methylations or acetylations can steadily spread to a tens kb of DNA region and the region can switch between the "all-unwrapped" and "all-wrapped" states, corresponding to whether the gene is expressed or kept silenced.[17,18] For comparison, in Fig. 2(d) we show the number of methylated histones, $N_{\rm M}$, versus time t. From Figs. 2(c) and 2(d), we see that, when the region is at the "high-M" state, most histones are in the wrapped conformations. This feature can also be noted from the results that, when the region is at the "high-A" state, most histones are in the unwrapped conformations (data not shown). It is also noted that, if the modification state is not stably in one state, the conformation of the chromatin is also not stable and each histone switches frequently between the wrapped and unwrapped conformations (data not shown). In other words, the two variables $N_{\rm M}$ and $N_{\rm W}$ have the same trend with time. These results thus show that the histone modification states determine the conformational states of nucleosomes.

Figure 3 represents the case that the state of the chromatin switches twice. From Fig. 3(a), we can see that nearly all of the nucleosomes are in the unwrapped conformation for $t \leq 30000$. Then, from $t = 3000$ to $t = 60000$, the histones are rapidly wrapped with a DNA strand and stay in the same conformation for a sufficiently long time. After that, nearly all of the nucleosomes become unwrapped again. These features can be clearly seen in Fig. 3(b), which shows the methylated histones as a function of time t. When $t = 31000$, the "high-A" state (low $N_{\rm M}$) suddenly changes to the "high-M" state and, correspondingly, the "all-unwrapped" state suddenly switches to the "all-wrapped" state; while at $t = 60000$ the "high-M" state changes back to the "high-A" state and, correspondingly, the "all-wrapped" state switches back to the "all-unwrapped" state.

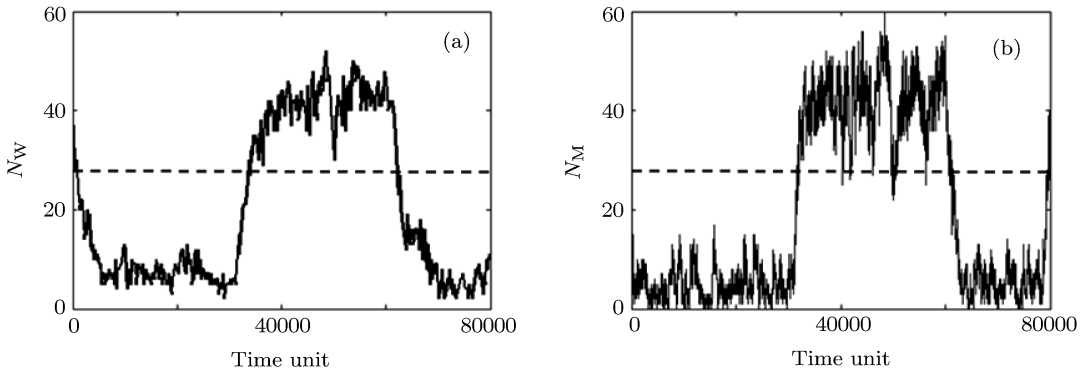


Fig. 3. (a) Number $N_{\rm W}$ of histones that are wrapped with a DNA chain obtained by using the Brownian dynamics model. (b) Number $N_{\rm M}$ of histones in the M state obtained by using the stochastic model. The dash line is the division of the "all-wrapped" state and "all-unwrapped" state (or division of the "high-M" state and "high-A" state).

Up to now, we have only studied the switching between two bistable states. As is known, another crucial feature of the chromatin is the inheritance which is involved in the DNA replication. Next, we study whether the "high-M" state or the "high-A" state and, correspondingly, the "all-wrapped" state or the "all-unwrapped" state maintains when DNA is replicated several times.

Upon DNA replication, the parental nucleosomes are believed to be partitioned randomly between the two daughter strands, and new nucleosomes are inserted or assembled to fill the gaps.[15] The real modification state of the inserted nucleosomes after replication is still unclear. Here, for simplicity, the new nucleosomes are assigned to be in the U state.

Figure 4(a) plots the rate of the whole region transited to the opposite state (i.e., from the "high-M" to "high-A" states or from the "high-A" to "high-M" states) during DNA replication as a function of the generation time for different values of F. The results are obtained with stochastic simulation. From the figure, we see that the transition rate decreases as F increases. When $F = 3.5$, the transition rate can reach a value close to the experimental data (about 10^{-3} transitions per cell generation) at the region that the generation time is larger than 1000. Thus, in the following calculations we take $F = 3.5$ and the generation time as 3000.

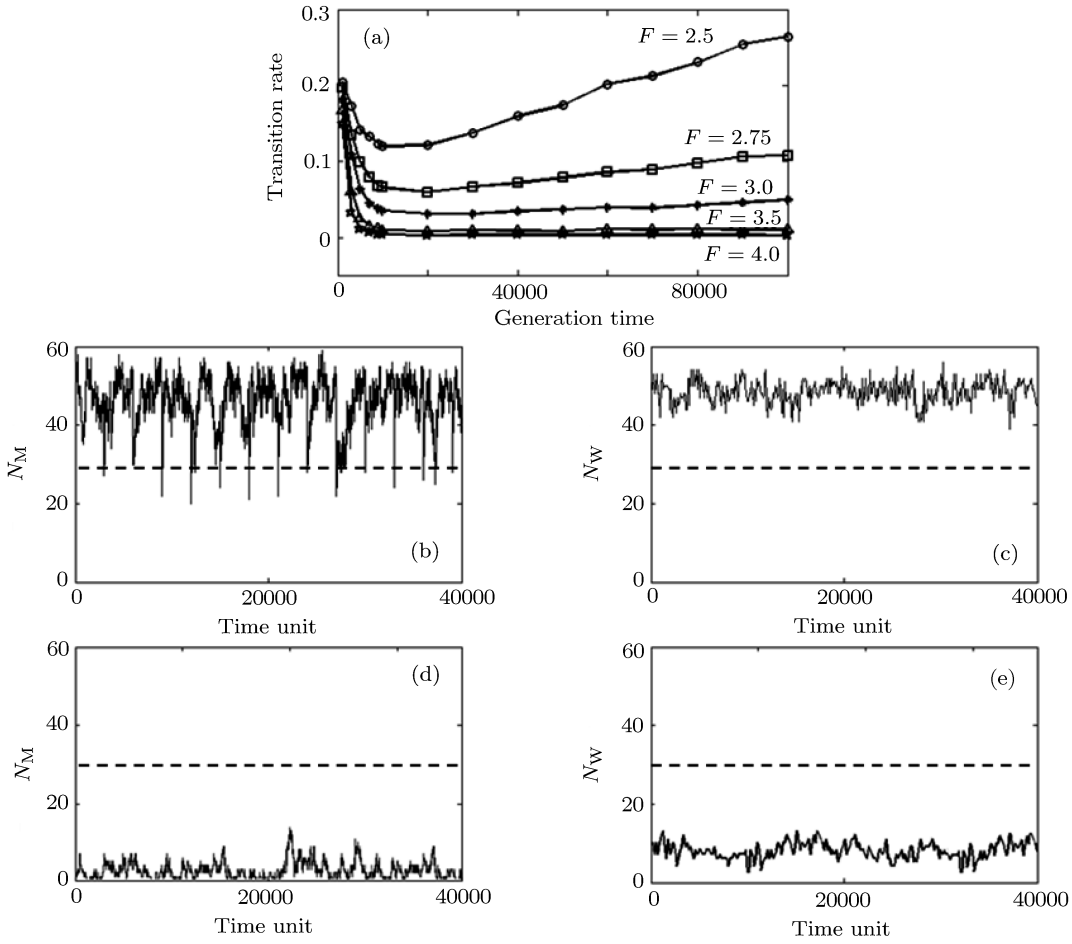


Fig. 4. (a) Transition rate as a function of generation time for different values of F. (b) Number $N_{\rm M}$ of histones in the high M state. (c) Number $N_{\rm W}$ of nucleosomes in the wrapped conformations. (d) Number $N_{\rm M}$ of histones in the "high-A" state. (e) Number $N_{\rm W}$ of nucleosomes in the wrapped conformations.

Figures 4(b) and 4(c) show two typical results by using both stochastic and Brownian dynamics simulations, where there are about 13 replication processes during the time period from 0 to 40000. Figure 4(b) corresponds to the results by using stochastic simulation, while Fig. 4(c) to the results by using a Brownian dynamics model. In the simulations, the DNA region is initially set to be in the "high-M" or "high-A" state and the DNA replication is considered to complete suddenly, i.e., the number of histones in the A or M state falls suddenly to about 20. We find that, upon each replication, nearly all of histones jump back to the "high-A" state or the "high-M" state, and stay at these states until the next replication (see Figs. 4(b) and 4(d)). Although the modification states have such jumps, the number of wrapped histones is just kept in one level, namely the "all-wrapped" or "all-unwrapped" state of the chromatin is kept unchanged (see Figs. 4(c) and 4(e)). This implies that, if the parent has an active or a silenced state in that DNA region, the daughter will be also in the same state. These results demonstrate that our model can explain some epigenetic phenomena.

4. Conclusion

We have numerically studied the interactions among an array of modified histones by using both stochastic and Brownian dynamics models. It is found that the modification state can determine the conformation of a histone-DNA complex. When a histone is in the A modified state, DNA is easily unwrapped from it, while when a histone is in the M modified state DNA becomes stably wrapped around it. A modified histone can also influence the modification state of other histones by the "cross-talking" effect. The stable "high-A" or "high-M" state and the stable "all-unwrapped" or "all-wrapped" state are obtained in our simulation. These

states will affect gene expression in various ways. Furthermore, the bistable states can inherit as a kind of epigenetic phenomenon. Thus, these models may be helpful for understanding the modification interactions of nucleosomes and the epigenetic phenomenon.

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