Chromatin Dynamics: Nucleosomes go Mobile through Twist Defects

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We study the spontaneous "sliding" of histone spools (nucleosomes) along DNA as a result of thermally activated single base pair twist defects. To this end we map the system onto a suitably extended Frenkel-Kontorova model. Combining results from several recent experiments we are able to estimate the nucleosome mobility without adjustable parameters. Our model shows also how the local mobility is intimately linked to the underlying base pair sequence.

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The genetic information of all higher organisms is organized in huge beads-on-a-chain arrays consisting of centimeters to meters of DNA wrapped around globular aggregates of so-called histone proteins. The basic unit of chromatin, the nucleosome, is a tiny $10 \times 5 \times 6$ nm sized spool composed of 147 base pairs (bps) DNA tightly wrapped around an octamer made from eight histone monomers. Each nucleosome is connected via a stretch of "linker" DNA to the next such protein spool. The wrapped DNA, being coiled in $\sim 1\frac{3}{4}$ turns of a left-handed helix with radius ~ 4.2 nm, is strongly distorted from its preferred straight ground state due to strong interactions with the histone octamer, namely, short range electrostatics (between the negatively charged DNA sugar-phosphate backbone and the positively charged octamer surface) and through extensive hydrogen bonding — both localized at 14 discrete interaction patches helically arranged along the octamer surface [1].

Higher order structures, from the 30 nm-chromatin fiber up to the highest level of DNA condensation, the fully folded chromosome, are designed to achieve a huge DNA volume fraction. They all rely on the significant stability of the nucleosome complex. On the other hand, fundamental life processes like transcription (making RNA offprints from the underlying DNA) and DNA replication seem to be in conflict with the picture of a stable nucleosome, as they are all performed by protein machines that track the DNA helix. The latter inevitably implies that every DNA bound obstacle (protein) has to be penetrated or even completely removed from its DNA target. In fact, the numbers are quite dramatic: A typical gene extends over hundreds of nucleosomes, each contributing 30–40k_BT net adsorption energy [2,3]. Also other mechanisms like the gene activation rely on regulatory protein binding to specific DNA sequences that are often covered by nucleosomes making them inaccessible.

A key to the understanding of these seemingly contradictory features might be the physical phenomenon of thermally driven nucleosome "sliding" along DNA (also called nucleosome repositioning) which has been repeatedly observed in well-defined *in vitro* experiments [4–6], reviewed in Ref. [7]. Spontaneous repositioning is strongly temperature dependent; at room temperature nucleosomes move a few tens of bps within an hour [8], with an apparent 10 bp step length [5]. However, recent experiments [6] indicate that, depending on the underlying DNA sequence, repositioning might also occur via local 1 bp steps. Despite clear evidence for repositioning the underlying mechanism has been the matter of long-standing controversy, especially due to the lack of any quantitative theoretical treatment of nucleosome statics and dynamics that has to rely on the detailed knowledge of the molecular structure and its underlying parameters.

Only very recently, since the documentation of the high resolution x-ray structure [1] and the presentation of other new experiments [2,3,6], has this become possible. First theoretical models of nucleosome repositioning [9,10] assume that it is based on the formation of DNA loop defects that form on either end of the nucleosomal DNA followed by their thermal diffusion around the octamer, similar to the de Gennes–Edwards reptation mechanism. This model seems to be successful in explaining the apparent 10 bps quantization of the nucleosome "jump" length [5] and it also reproduces the observed diffusion constants. However, the more local 1 bp-step mechanism observed in Ref. [6] cannot be understood within this model. This led us here to consider an alternative mechanism: twist diffusion. The carrier of motion in this case is a twist defect that contains one missing or one extra bp.

Interestingly twist defects have been observed in the high resolution crystal structure of the core particle (the octamer plus wrapped DNA) [1]. In that study the core particles were reconstituted from histones and DNA of 146 bp assuming that this would be its optimal length in the crystal. However, the latter turned out to be 1 bp longer, i.e., 147 bp. It was found that the missing bp of the 146 bp DNA was not localized at its terminus but instead at a 10 bp stretch close to the dyad axis [cf. Fig. 4(d) in Ref. [1]]. That twist defect allows the DNA termini of adjacent particles in the crystal to come close in order to mimic a bp step. We suggest that this gives an upper bound for the cost of a single twist defect, namely, the stacking energy of the blunt ends ~ 10–$20k_BT$ [11].

In order to model the twist-diffusion mechanism we map the nucleosomal DNA on a Frenkel-Kontorova (FK) chain of particles connected by harmonic springs in a spatially periodic potential (cf. Fig. 1). The original FK model was introduced more than 60 years ago to describe the motion of dislocations in crystals [12]. In the mean time variants of this model were applied to many different problems including charge density waves [13], sliding friction [14,15], ionic conductors [16,17], chains of coupled Josephson junctions [18], and adsorbed atomic monolayers [19,20]. Here, in the context of DNA adsorbed on the octamer, the beads represent the base pairs. The springs in between have an equilibrium distance $b = 0.34$ nm and a constant that reflects the coupled DNA twist-stretch elasticity. Specifically

$$E_{\text{elastic}}(\{x_n\}) = \sum_k C\left(\frac{x_{k+1} - x_k}{b} - 1\right)^2. \quad (1)$$

Here the conformation of the wrapped DNA is given by the set $\{x_n\}$ where x_n is the position of the nth bp measured along the helical backbone; $C \simeq (70\text{–}100)k_BT$ is the combined twist and stretch spring constant including the (here unfavorable) twist-stretch coupling [21], and the summation goes over all bp associated with the wrapped DNA. In addition there is the external potential of the 14 contact points to the octamer with neighboring points being 10 bp apart [1] that we model as follows:

$$E_{\text{ads}}(\{x_n\}) = -U_0 \sum_k \sum_{l=1}^{14} \left[\left(\frac{x_k - 10bl}{a}\right)^2 - 1\right]^2 \times \theta(a - |x_k - 10bl|), \quad (2)$$

with θ being the Heaviside step function. The two parameters of the external potential, its depth U_0 and its width a, can be estimated as follows. U_0 represents the pure adsorption energy per point contact which follows from competitive protein binding [2] to be of order $6k_BT$. The other parameter, a, can be estimated from

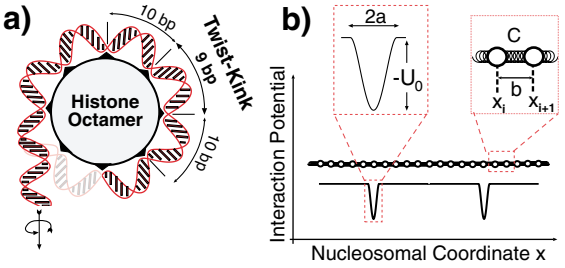

FIG. 1 (color online). The twist-diffusion mechanism for nucleosome repositioning. (a) A concerted translational and rotational motion of DNA leads to injection of twist defects (kinks) which migrate between the octamer adsorption sites (black triangles) leading to a "creep" motion of DNA. (b) The corresponding Frenkel-Kontorova model for twist diffusion and its characteristic parameters (see text for details).

the fluctuations of the DNA in the crystal measured by the B factor [cf. Fig. 1(b) in [1]] at different nucleosome positions. The ratio of DNA helix fluctuations $R_{\text{fluct}} = \langle x^2_{\text{middle}}\rangle / \langle x^2_{\text{bond}}\rangle \approx 3$ at positions between the binding sites and at the bound sites is a measure of DNA localization. Using a quadratic expansion of Eq. (2) one finds from a straightforward normal mode analysis that $a = (5U_0/[(R_{\text{fluct}} - 1)C])^{1/2} b \sim b/2$, i.e., the adsorption regions lead to a strong localization of the DNA. Knowing all involved parameters the total energy of the DNA chain confined in the nucleosome can be written down

$$E_{\text{tot}} = E_{\text{elastic}} + E_{\text{ads}} + E_{\text{sd}}. \quad (3)$$

The last term E_{sd} is the sequence dependent part of the total energy which we will neglect first. In the following we study the mechanism for thermal motion of DNA governed by E_{tot}. Generally two scenarios are possible: (i) the injection of a kink (1 bp missing) or antikink (1 additional bp) at either nucleosome end and (ii) the generation of kink-antikink pairs inside the nucleosome. Since the second mechanism is energetically roughly twice as costly than the first one, we will focus here on the (anti)kink injection mechanism only.

How and how fast does the kink step around the nucleosome? Because of the strong DNA localization at the binding sites ($a/b < 1$) for a realistic range of parameters U_0 and C a given kink is localized either between two adsorption positions, i.e., smeared out over 10 bp (denoted by the K_{10} state), or between three of them, i.e., smeared out over 20 bp (the K_{20} state). It is obvious that the motion of a (anti)kink will consist of an alternation between K_{10} and K_{20} states similarly to an earthworm creep motion. To model this process we introduce the effective kink coordinate x_K describing the coordinate of that bp that goes from being pinned to being depinned during a single kink step, so that $x_K \approx 0$ and $x_K \approx b/2$ correspond to K_{10} and K_{20}, respectively, whereas $x_K \approx b$ means that the kink moved by 1 bp step. The Peierls-Nabarro potential experienced by the kink is then given by $U_{\text{PN}}(x_K) = C_{\text{eff}}(x_K/b - 1/2)^2 - U_0(x_K^2/a^2 - 1)^2\theta(a - x)$ for $0 < x_K < b/2$ and $U_{\text{PN}}(x_K) = U_{\text{PN}}(b - x_K)$ for $b/2 \le x_K < b$. Here $C_{\text{eff}} = (2/10 \pm 1)C$ with $-$ referring to a kink and $+$ to an antikink. Depending on the ratio of parameters U_0 and C, the state K_{20} corresponds to a local minimum or maximum of U_{PN}, whereas K_{10} is always stable for the relevant parameter range. The rate for the kink step process is then given by the expression $f_{\text{step}} = k_BTj_0/b^2\zeta_{\text{eff}}$ with $j_0^{-1} = (\int_0^1 e^{-U_{\text{PN}}(sb)/k_BT} ds)(\int_0^1 e^{+U_{\text{PN}}(sb)/k_BT} ds)$ and $\zeta_{\text{eff}} = (4\pi^2/10b)\mu_{\text{spin}}$, the effective kink friction constant. Here $\mu_{\text{spin}} = 1.3 \times 10^{-20} Ns$ is roughly the rotational friction for a single base step [22]. To determine the rate at which twist defects are formed at the entry/exit points of the DNA one can now use an argument similar to the one presented in Ref. [9]: The ratio of the lifetime t_{life} of a

kink to the time interval t_{inj} between two kink injection events at the end of the wrapped DNA portion equals the probability to find a defect on the nucleosome, i.e., $t_{\text{life}}/t_{\text{inj}} \simeq N_{\text{site}} e^{-U_{\text{Kink}}/k_B T}$. Here $N_{\text{site}} = 13$ denotes the number of possible positions of the defect between the 14 binding sites and $U_{\text{Kink}} \simeq C/10$ is the energetic cost for a single kink (cf. above).

How is the average lifetime t_{life} of a defect related to t_{step}, the typical time needed for one step? This can be determined from the mean first passage times τ_{left} and τ_{right} for a defect that forms, say, at the left end to leave the nucleosome at the same or at the other end, respectively. From Ref. [23] one finds $\tau_{\text{left}} = (25/6)t_{\text{step}}$ and $\tau_{\text{right}} = 28t_{\text{step}}$. Furthermore, the probability to leave at the left end is $p_{\text{left}} = 12/13$ and at the right end $p_{\text{right}} = 1/13$ [23] which gives the lifetime as the weighted average $t_{\text{life}} = 6t_{\text{step}}$. Only a fraction p_{right} of the defects reaches the other end and will lead to a repositioning step, i.e., the time of a 1 bp diffusion step of the nucleosome along the DNA is given by $T = t_{\text{inj}}/p_{\text{right}}$. Putting all this together we arrive at $T \simeq 6b^2 \zeta_{\text{eff}} j_0^{-1}/k_B T \exp(C/10k_B T)$. For realistic parameter values $C = 100k_B T$, $U_0 = 6k_B T$ and $R_{\text{fluct}} = 3$ we find $T \simeq 10^{-3}$ s implying a nucleosome diffusion constant $D = 580\ \text{bp}^2/\text{s} = 6.6 \times 10^{-17}\ \text{m}^2/\text{s}$. Note that $U_{\text{Kink}} \simeq 9k_B T$ for K_{10} and $\simeq 11k_B T$ for K_{20}.

Hence we find repositioning rates that are orders of magnitude faster than the ones observed in experiments [5]. Even worse, the experimental observation of an apparent 10 bp jump length [5] seems to be inconsistent with our predictions. We show now how these facts can be explained by the existence of additional barriers with a 10 bp periodicity. To do so we have to extend our simple model to deal with the quenched disorder stored in the DNA bp sequence. The sequence dependent anisotropic bendability, i.e., the propensity of DNA to bend in different directions with different elastic constants, turns out to be essential. It has been known for a long time [24,25] that (A/T) rich dinucleotide steps (dns) prefer to face the octamer in the minor groove (i.e., at the octamer contact points) whereas (G/C) rich dns prefer to face the octamer in the major groove (i.e., between contact points). This reflects different propensities of the dinucleotides to widen or compress towards the DNA minor groove. To incorporate these anisotropic effects into our model we first note that the bending state of the DNA molecule is fully constrained by its helical path on the octamer surface. Moving a DNA sequence via twist diffusion by a few bp (< 10 bp) along that path changes the relative rotational setting of the bent DNA with respect to its preferred bending direction causing an energetic penalty, whereas a motion by 10 bp restores the initial rotational setting. We address this by introducing a 10 bp periodic "bending field" $F_{\text{bend}}(x) = -\cos[2\pi x/(10b)]$ attached to the octamer surface. We assume the DNA sequence to couple linearly to that field through "bending charges" q_k attached to each of the dns. This gives us finally the third term in Eq. (3):

$$E_{\text{sd}} = \sum_k q_k F_{\text{bend}}(x_k) + m_k. \tag{4}$$

In addition to the anisotropic term we also introduced here the isotropic bending parameters m_k to include isotropic flexibility effects (which become important when the q_k's vanish or average out). The summation involved is again over all base pairs incorporated in the nucleosome. q_k and m_k both have units of energy and can be extracted from competitive protein binding experiments [25] for each of the 10 dns (AA, AT, GC, ...). To obtain a rough estimate we distribute the dns into three classes: (1) (G/C) containing dns, (2) (A/T) containing dns, and (3) mixed dns (like AG, CT, etc.) and treat the dns in each class as identical. Using the available experimental data [25,26] we then arrive at $q_{\text{G/C}} \approx 95$, $q_{\text{A/T}} \approx -85$, $q_{\text{mixed}} \approx 0$ and $m_{\text{G/C}} \approx 20$, $m_{\text{A/T}} \approx -3$, $m_{\text{mixed}} \approx 7$, where all energies are in cal/mol per dns.

It turns out that the nucleosome mobility depends strongly on the underlying bp sequence. When shifting the position of all beads by l bp steps, $x_k \to x_k + lb$, we find $E_{\text{sd}}(l) = (A/2)\cos(2\pi l/10 - \phi)$ to vary as a cosine function of l with phase ϕ and amplitude A determined by the DNA sequence, which is assumed to be appropriately periodic here. Arranging G/C and A/T tracts properly and taking the sequence dependent q and m values given above we can easily reach amplitudes A (i.e., barriers to repositioning) that exceed 10–12 kcal/mol. A very effective sequence arrangement called the "TG" sequence which leads to a strong nucleosome stability and localization was experimentally constructed in Ref. [25] by putting G/C tracts around positions $k = 0, 10, 20, \ldots$, and A/T tracts around $k = 5, 15, 25, \ldots$. In our picture

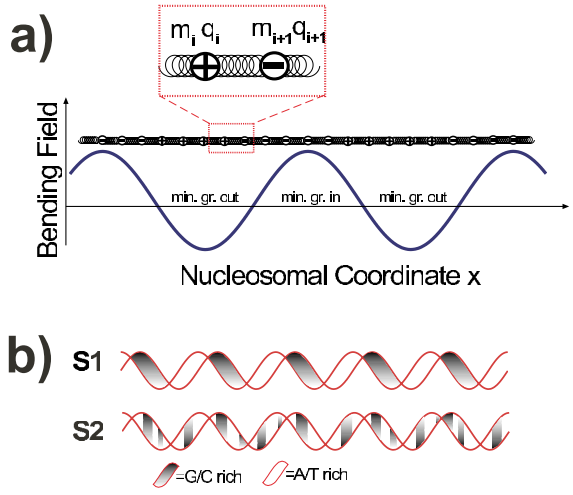

FIG. 2 (color online). The extended Frenkel-Kontorova model includes effects from anisotropic bp sequences. (a) DNA sequences couple additionally to an octamer-fixed "bending field" through the anisotropic bending parameters q_i ("bending charge"). (b) Two sequences with extremely different mobilities. S1: highly anisotropic, 10 bp phased ("TG"-like) sequence with $D_{\text{sd}} \approx 10^{-4}$–$10^{-5}\ \text{bp}^2/\text{s}$. S2: random sequence corresponding to $> 95\%$ of the genome with $D_{\text{sd}} \approx 10^2\ \text{bp}^2/\text{s}$.

this means to put the "bending charges" q along the DNA such that they couple favorably to the bending field F_{bend} for a distinct rotational setting whereas a 5 bp shift is extremely costly (cf. Fig. 2). The 5S-RNA sequence which was used in most nucleosome mobility experiments shows also the effect of an optimal rotational setting. It is less pronounced than in the TG case, yet it is still detectable. More involved theoretical computations relying on molecular sequence dependent deformability parameters [27] reveal barriers $A \approx 5$–6 kcal/mol for this particular sequence. The sequence dependent barrier height A exponentially suppresses the bare (sequence independent) diffusion constant D obtained above leading to the sequence dependent diffusion constant D_{sd}:

$$D_{\text{sd}} = DI_0^{-2}(A/2k_BT) \approx \frac{\pi j_0 A}{12\zeta_{\text{eff}}} e^{-(A+C/10)/k_BT}, \quad (5)$$

with I_0 being the modified Bessel function.

Equation (5) predicts that mobility experiments with highly anisotropic sequences like TG (instead of the standard "5S-RNA") would find hardly any appreciable repositioning on the 1 h time scale *if* it would be solely mediated via twist defects ($D_{\text{sd}} = 10^{-6}$–$10^{-7} \times D = 10^{-4}$–$10^{-5}$ bp^2/s). The typical path for a nucleosome to escape from such a rotational trap goes very likely via the previously considered loop formation mechanism [9,10] that allows "tunneling" over sequence barriers, thus dominating over twist diffusion for extremely anisotropic sequences. An experimental test for this prediction would be to increase the free DNA segment length which in this regime should strongly enhance the loop mediated mobility [10], whereas it would leave the twist diffusion unaffected. Going to the other extreme, in the most relevant case of random isotropically bendable sequences which make up more than 95% of the eucaryotic genome one should observe that the twist-diffusion mechanism is strongly enhanced by 2–3 orders of magnitude as compared to the *in vitro* measurements on 5S-RNA.

In conclusion the following picture is implied: On physiological time scales the majority of genomic nucleosomes seems to be intrinsically highly mobile. However, only a small fraction ($< 5\%$) of all nucleosomes has strongly reduced mobility due to anisotropic DNA sequences which they populate. We speculate that only the latter require the action of active (adenosin triphosphate consuming) remodeling mechanisms [28] making them hot spots and switching elements for global chromatin rearrangements.

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[1] K. Luger, A.W. Mäder, R. K. Richmond, D. F. Sargent, and T. J. Richmond, Nature (London) **389**, 251 (1997).
[2] K. J. Polach and J. Widom, J. Mol. Biol. **254**, 130 (1995); **258**, 800 (1996).
[3] B. D. Brower-Toland, C. L. Smith, R. C. Yeh, J. T. Lis, C. L. Peterson, and M. D. Wang, Proc. Natl. Acad. Sci. U.S.A. **99**, 1960 (2002).
[4] P. Beard, Cell **15**, 955 (1978); C. Spadafora, P. Oudet, and P. Chambon, Eur. J. Biochem. **100**, 225 (1979).
[5] S. Pennings, G. Meersseman, and E. M. Bradbury, J. Mol. Biol. **220**, 101 (1991); Proc. Natl. Acad. Sci. U.S.A. **91**, 10 275 (1994); G. Meersseman, S. Pennings, and E. M. Bradbury, EMBO J. **11**, 2951 (1992).
[6] A. Flaus and T. J. Richmond, J. Mol. Biol. **275**, 427 (1998); J. M. Gottesfeld, J. M. Belitsky, C. Melander, P. B. Dervan, and K. Luger, J. Mol. Biol. **321**, 249 (2002).
[7] H. Schiessel, J. Phys. Condens. Matter **15**, R699 (2003).
[8] These experiments are performed with nucleosomes depleted of linker histones. The latter are known to glue the entering and exiting DNA strands together suppressing repositioning at physiological ionic strength, cf. [7].
[9] H. Schiessel, J. Widom, R. F. Bruinsma, and W. M. Gelbart, Phys. Rev. Lett. **86**, 4414 (2001); **88**, 129902 (2002).
[10] I. M. Kulić and H. Schiessel, Biophys. J. **84**, 3197 (2003).
[11] R. L. Ornstein, R. Rein, D. L. Breen, and R. D. Macelroy, Biopolymers **17**, 2341 (1978).
[12] J. Frenkel and T. Kontorova, Zh. Eksp. Teor. Fiz. **8**, 1340 (1938); J. Phys. (Moscow) **1**, 137 (1939).
[13] L. M. Floria and J. J. Mazo, Adv. Phys. **45**, 505 (1996).
[14] O. M. Braun, T. Dauxois, M.V. Paliy, and M. Peyrard, Phys. Rev. Lett. **78**, 1295 (1997).
[15] T. Strunz and F.-J. Elmer, Phys. Rev. E **58**, 1601 (1998).
[16] L. Pietronero, W. R. Schneider, and S. Strässler, Phys. Rev. B **24**, 2187 (1981).
[17] S. Aubry, J. Phys. (France) **44**, 147 (1983).
[18] S. Watanabe, H. S. J. van der Zant, S. H. Strogatz, and T. P. Orlando, Physica (Amsterdam) **97D**, 429 (1996).
[19] W. Uhler and R. Schilling, Phys. Rev. B **37**, 5787 (1988).
[20] H. Schiessel, G. Oshanin, A. M. Cazabat, and M. Moreau, Phys. Rev. E **66**, 056130 (2002).
[21] R. D. Kamien, T.V. Lubensky, P. Nelson, and C. S. O'Hern, Europhys. Lett. **38**, 237 (1997); J. F. Marko, Europhys. Lett. **38**, 183 (1997).
[22] C. Levinthal and H. Crane, Proc. Natl. Acad. Sci. U.S.A. **42**, 436 (1956); P. Nelson, Proc. Natl. Acad. Sci. U.S.A. **96**, 14 342 (1999).
[23] N. G. van Kampen, *Stochastic Processes in Physics and Chemistry* (North-Holland, Amsterdam, 1992).
[24] S. C. Satchwell, H. R. Drew, and A. A. Travers, J. Mol. Biol. **191**, 659 (1986).
[25] T. E. Shrader and D. M. Crothers, Proc. Natl. Acad. Sci. U.S.A. **86**, 7418 (1989); J. Mol. Biol. **216**, 69 (1990).
[26] S. Cacchione, M. A. Cerone, and M. Savino, FEBS Lett. **400**, 37 (1997).
[27] S. Mattei, B. Sampaolese, P. De Santis, and M. Savino, Biophys. Chem. **97**, 173 (2002); C. Anselmi *et al.*, Biophys. J. **79**, 601 (2000).
[28] Y. Lorch, M. Zhang, and R. D. Kornberg, Cell **96**, 389 (1999).