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MINIREVIEW

The dynamics of the nucleosome: thermal effects, external forces and ATP

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With nucleosomes being tightly associated with the majority of eukaryotic DNA, it is essential that mechanisms are in place that can move nucleosomes 'out of the way'. A focus of current research comprises chromatin remodeling complexes, which are ATP-consuming protein complexes that, for example, pull or push nucleosomes along DNA. The precise mechanisms used by those complexes are not yet understood. Hints for possible mechanisms might be found among the various spontaneous fluctuations that nucleosomes show in the absence of remodelers. Thermal fluctuations induce the partial unwrapping of DNA from the nucleosomes and introduce twist or loop defects in the wrapped DNA, leading to nucleosome sliding along DNA. In this minireview, we discuss nucleosome dynamics from two angles. First, we describe the dynamical modes of nucleosomes in the absence of remodelers that are experimentally fairly well characterized and theoretically understood. Then, we discuss remodelers and describe recent insights about the possible schemes that they might use.

Introduction

We are in the middle of a soft revolution in our understanding of how chromatin structure, and hence gene expression in eukaryote organisms, is regulated. Progress is taking place on many fronts: the positioning of nucleosomes [1]; the clarification of the role of histone modifications [2]; and the resolution of the structure of the large complexes involved in regulation, including polymerases such as Pol II [3], the mediator complex [4] or the chromatin remodelers [5]. These advances have become possible as a result of the concerted efforts of molecular and structural biologists, physicists and bioinformaticians.

The present minireview focuses on a mechanistic view of the regulation of nucleosomes. The biophysical properties of the nucleosome, which is the basic repeat unit of the chromatin complex, can currently be probed by diverse experimental means (e.g. single-molecule analysis or imaging with fluorescence energy transfer; FRET). Here, we focus on a theoretical understanding of some of the experimental observations made on nucleosome mechanics that allow for a modeling approach based on statistical physics methods.

In a living cell, nucleosomes undergo various dynamic processes that change their positions on DNA. We distinguish here between mechanisms intrinsic to this histone–DNA complex, which therefore allow for an excitation by thermal energy alone, and those processes by which nucleosomal positions are actively regulated by molecular interactions with chromatin

Abbreviations
FRET, fluorescence energy transfer; WLC, wormlike chain.

remodelers, and which therefore consume energy in the form of ATP. As is often the case in biology, the thermal equilibrium processes form a basic repertoire that more specific energy-consuming processes can then exploit. We therefore expect that an understanding of the former will ultimately help us to better understand the more specific processes, which are generally those of prime interest to molecular biologists.

Autonomous nucleosome dynamics

With three-quarters of eukaryotic DNA being wrapped into nucleosomes, the question arises as to how DNA-binding proteins can bind to their target sequence if this sequence happens to be located inside a wrapped DNA portion. Spontaneous fluctuations of the nucleosomes might allow transient access to any target sequence. This is possible via two modes: nucleosome breathing, where the DNA partially unwraps from the protein core, and nucleosome sliding, where the octamer moves as a whole along the DNA. Below, we discuss these two modes separately.

Unwrapping

Nucleosome breathing

Here, we try to answer two questions. How do DNA-binding proteins gain access to their specific target site if this site happens to be buried in the wrapped DNA portion of a nucleosome? How can we obtain a quantitative estimate of the energetics involved in the DNA wrapping in nucleosomes? Both questions have been answered in experiments, as discussed below. Before going into the details of these experiments, we note that it is now known, from the nucleosome crystal structure [6], that there are 14 regions where the wrapped DNA contacts the octamer surface, located where the minor groove of the DNA double helix faces inwards towards the surface of the octamer. At each contact region, there are several direct hydrogen bonds, as well as positive charges that attract the phosphates of the DNA backbones. To bind at those sites, however, DNA has to pay a high price. An estimate for this price follows from the wormlike chain (WLC) model [7]. This estimate is not very precise because one cannot expect that the WLC model works very well for such strong DNA bending as is observed in the nucleosome, although at least it will provide an approximate idea of the energy involved. In a nucleosome, 127 bp of the DNA are bent around the octamer (10 bp at each terminus are essentially straight) [6], leading to an elastic energy

$$\frac{E_{\text{elastic}}}{k_{\text{B}}T} = \frac{l_{\text{P}} l}{2R_0^2} \tag{1}$$

Here, l_{P} is the DNA persistence length, which is ~ 50 nm; l is the bent part of the wrapped DNA, $\sim 127 \times 0.34$ nm $= 43$ nm; and R_0 is the radius of curvature of the centerline of the wrapped DNA, which is ~ 4.3 nm. This leads to a bending energy on the order of 58 $k_{\text{B}}T$. Note that, in Eqn (1), we assume isotropic DNA bending behavior but that nucleosomes are often localized at nucleosome-positioning sequences with a preferred bending direction and a lower bending modulus. If we nevertheless use this estimate, we find that the binding energy of the 14 sites together must exceed 58 $k_{\text{B}}T$, and that it should exceed it by a substantial amount so that the nucleosome is stable and does not fall apart immediately.

Polach and Widom [8] demonstrated that a nucleosome is a dynamic structure, with parts of its DNA spontaneously unwrapping from either of its ends. They proposed that this mechanism gives DNA-binding proteins access to target sequences if they happen to be located inside a nucleosome. Indeed, the experiment was based on measuring the accessibility directly for certain proteins. We discuss this method below, but we first review newer measurements that detect the unwrapping dynamics more directly by the use of FRET. To do so, a donor and an acceptor dye were attached on the DNA and on the octamer [9,10] or both were attached on the DNA [11–17]. In all the experiments, the donor and acceptor were positioned in such a way that they were close to each other for a completely wrapped nucleosome, leading to a FRET signal. For sufficiently unwrapped DNA, the distance between donor and acceptor increased, reducing the FRET efficiency. A shift in FRET populations with DNA sequence variation [12–15], position of the dyes [13,17] and histone acetylation [15] was detected. Typical times observed in the experiments are 250 ms for the wrapped state interrupted by 10–50 ms long episodes where the DNA is unwrapped [10]. Koopmans *et al.* [17] identified different states of unwrapping that, depending on the position of the dyes, have a different equilibrium constant, K_{eq}, defined as the ratio of the unwrapped to the wrapped fraction. When the labels are positioned to detect unwrapping at the end of the wrapped portion, $K_{\text{eq}} \approx 0.19$–$0.37$, whereas, 27 bp deeper into the nucleosome, a smaller value of $K_{\text{eq}} \approx 0.07$ is found.

Not much theoretical work has been carried out to allow a clearer quantitative picture of these observations. A geometrical model of the nucleosome was used for comparison with the experimentally observed

FRET efficiencies to identify states with an intermediate unwrapping [16]. However, an even more detailed theoretical analysis of the geometry and elasticity of the various unwrapping states is necessary to capture the experimental data quantitatively (L. Lenz, P. Prinsen & H. Schiessel, unpublished data). A theoretical analysis of the dependence of the breathing dynamics on the length of unwrapped DNA has been presented previously [19].

FRET experiments suffer still from the problem that they do not give sufficiently systematic sets of quantitative data. For example, one would like to have data for fluorescence energy efficiencies for a large number of different dye positions inside the nucleosome. In addition, and even though this method promises to give relatively direct insight into the geometries of the various unwrapping states, the real question that requires an answer is how much the presence of a nucleosome hinders the access of DNA-binding proteins to their target sequence. In these respects, the older approach discussed below has advantages over the FRET-based approaches.

The idea is to measure the binding of a protein to its specific DNA-binding site. This binding site is sterically not accessible as long as it is wrapped inside the wrapped portion (Fig. 1A). From time to time, the DNA spontaneously unwraps from either end of the wrapped portion. If it unwraps far enough to open the binding site of the protein, there is a window of opportunity for this protein to bind to its site. It is expected that the probability for having the binding site temporarily exposed decreases with the distance from the closest terminus of the wrapped portion and is smallest in the center of the wrapped portion at the nucleosomal dyad. This was indeed measured by Polach and Widom [8] using restriction enzymes, see also [20–23]. As long as the nucleosome is sufficiently wrapped, it is not possible for restriction enzymes to bind as a result of steric hindrance. Once the nucleosome unwraps its DNA far enough beyond the binding site of the enzyme, the enzyme can bind and then either unbind again or cut the DNA at that particular site. The rate at which the DNA degraded into the smaller segments over time was measured and compared with the rate of DNA cutting in a solution of naked DNA under identical conditions (except for the histone proteins being absent). In the latter case, DNA is cut much faster because the DNA-binding site does not first need to be exposed by unwrapping from the octamer. By comparing the naked DNA set-up with the one with the nucleosome, the probability of that particular binding site being open was deduced.

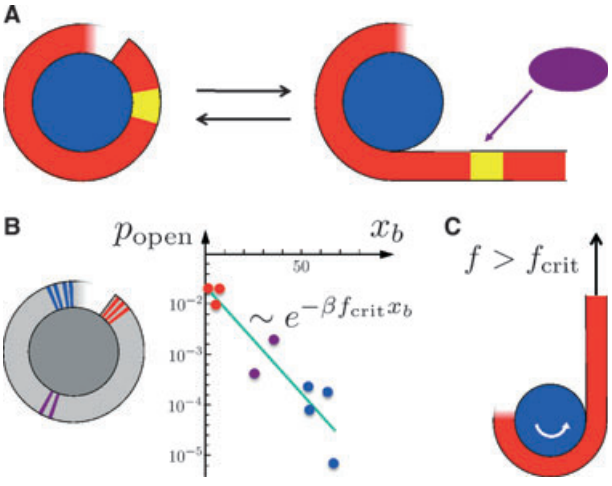

Fig. 1. (A) Nucleosome breathing: the spontaneous unwrapping of DNA from the octamer gives DNA-binding proteins access to the target sites. (B) Probability p_{open} to find the target site open as a function of its position x_{b} inside the nucleosome (bp). This experiment allows the effective adsorption energy f_{crit} to be determined. (C) Unwrapping of a nucleosome with a force $f > f_{\mathrm{crit}}$.

More specifically, in both set-ups, an exponential decay of the intact DNA over time is observed, with a rate k_{nuc} for the nucleosome and with a rate k_{naked} for the naked DNA. The probability that the binding site is open is then given by $p_{\mathrm{open}} = k_{\mathrm{nucl}}/k_{\mathrm{naked}}$; the detailed conditions under which this statement is true are reported in Prinsen & Schiessel [24]. In Fig. 1B, we show the result of the experiment [8], namely p_{open} for different sites x_{b} for restriction enzymes starting close to the entrance at $x_{\mathrm{b}} = 1$ bp and going close to the middle of the wrapped portion at $x_{\mathrm{b}} = 74$ bp. Note that the accessibility is greatly reduced for binding sites located in wrapped portions of the nucleosome, even for binding sites close to the terminus. This indicates that a substantial amount of DNA needs to be unwrapped beyond the actual binding site because the enzyme needs sufficient room for binding and cutting. Moreover, because the data points lie approximately along a line and p_{open} is plotted on a logarithmic scale, it follows that the probability decays exponentially towards the middle of the wrapped portion, $p_{\mathrm{open}} \sim \mathrm{e}^{-\beta f_{\mathrm{crit}} x_{\mathrm{b}}}$ where f_{crit} denotes the adsorption energy per length. The fit is best for $f_{\mathrm{crit}} = 1.2$ pN. In terms of the thermal energy, f_{crit} can be rewritten as $f_{\mathrm{crit}} = 0.3\ k_{\mathrm{B}}T{\cdot}\mathrm{nm}^{-1}$, from which follows a net adsorption energy of the total amount of DNA on the order of: $E_{\mathrm{net}} = f_{\mathrm{crit}} \times 50\ \mathrm{nm} \approx 15\ k_{\mathrm{B}}T$ [24].

This is an unexpectedly small number, especially taking into account the fact that we calculated above, in Eqn (1), that the pure adsorption energy, E_{elastic}, is

approximately four-fold larger. This means that nature must have tuned the pure adsorption energy E_{ads} such that its value is close to $E_{elastic}$, namely:

$$E_{net} = E_{ads} - E_{elastic} \approx 15\, k_B T \quad (2)$$

with $E_{ads} \approx 75\, k_B T$ and $E_{elastic} \approx 60\, k_B T$. The net energy per sticking site is thus just on the order of one $k_B T$, even though the pure adsorption energy is approximately five-fold and the elastic energy is approximately four-fold larger. As an explanation, if the net adsorption energy were much larger, the nucleosome would hardly ever show spontaneous breathing and thus the binding sites inside the nucleosomal DNA would not be accessible. By tuning the adsorption energy of the binding sites to be close to mechanical cost per 10 bp, the nucleosome is a highly dynamic structure that is accessible to DNA-binding proteins.

Being so dynamic, however, might come at a cost; the nucleosome might not be very stable and easily fall apart. This is especially the case if a protein binds at a DNA-binding site that is located deep inside the nucleosome. Once the protein is bound, the nucleosome cannot rewrap, although it might easily unwrap completely and disintegrate. Another problem occurs when a nucleosome comes under tension. This can easily happen inside the nucleus where many motor proteins act incessantly. However, as we shall see below, the nucleosome turns out to be much more stable than expected from Eqn (2) and this can be explained to a large extent by the two-turn spool geometry of the nucleosomal DNA.

Force-induced nucleosome unwrapping

The breathing experiment [8] predicts the force that is necessary to peel the DNA off the nucleosome (Fig. 1C). This force equals the ratio of the net adsorption energy, Eqn (2), and the wrapped length, 50 nm:

$$f_{crit} \approx \frac{15\, k_B T}{50\,\text{nm}} = 1.2\,\text{pN} \quad (3)$$

In other words, the critical force beyond which the nucleosome should become unstable is just the net adsorption energy per length that we estimated above, which we termed f_{crit}, anticipating its role as a critical force for unwrapping.

One of the first experiments to study the unwrapping of nucleosomes was performed in 2002 [25]; subsequently, additional experiments have been reported [26–30]. The experiment was performed on a DNA chain with 17 nucleosomes that were localized on well-defined positions via nucleosome-positioning sequences. One end of the DNA molecule was attached to a bead that was held in an optical trap, whereas the other end was attached to a coverslip that could be moved to stretch the nucleosomal array. It was observed that, when the end-to-end distance is increased, the force rises slowly at first, and then sharply. Once a certain force was reached, the force–extension curve showed a sawtooth pattern featuring 17 drops in the curve. After the 17 rupture events, the force–extension curve of the naked DNA chain was attained. Obviously, the 17 peaks represent the unwrapping events of the 17 nucleosomes. From the shift of the curve to the right at each step, one can estimate the length that is liberated, namely ~ 80 bp corresponding to one turn of DNA inside the nucleosome. The interpretation of this observation is that the first three-quarter turn must have already unwrapped earlier, whereas the distinct rupture events signal the complete unwrapping of the last turns in the nucleosomes. Importantly, the unwrapping of the last turns occurs sequentially (i.e. one nucleosome at a time) and not in parallel. In addition, the forces at which the nucleosomes unwrap are ~ 25 pN, which is 20-fold larger than expected. These two features clearly hint at a kinetic barrier underlying the unwrapping. Given sufficient time, the nucleosomes unwrap at much smaller forces but, because the array is stretched at a finite rate (e.g. 28 nm·s^{-1} for the 25 pN unwrapping peaks), the nucleosomes only jump over the barrier once much higher forces have built up.

To learn more about the barrier, dynamic force spectroscopy [31] was performed, where many nucleosomal arrays were stretched with given pulling rates r_f, increasing the force linearly in time t, $f = r_f t$, and a distribution of rupture forces was determined by combining the rupture events of all 17 nucleosomes. If the nucleosomes unwrap completely independently from each other, the distribution of forces of a 17 nucleosome chain should be identical to the distribution obtained from a series of experiments performed on single nucleosomes, notwithstanding the fact that earlier rupture events take place at typically smaller force values than later rupture events. The experimental data indicated a barrier height of ~ 33–$35\, k_B T$ and it was suggested that this energy is focused onto two binding sites that stabilize the last DNA turn [25]. This number, however, is in serious conflict with the estimate obtained above, indicating that the total net adsorption energy is $\sim 15\, k_B T$ (Eqn 2).

It was previously demonstrated [32] that the barrier could simply result from the underlying geometry of the nucleosome and the elasticity of the DNA. There is thus no need to assume that the barrier energy is

focused into two strong binding sites, as previously considered [25]. This follows from a model where the DNA is represented by a WLC under an external tension, f, and the histone octamer as a cylinder around which a section of the DNA is wrapped (Fig. 2). The total energy of the nucleosome under tension then has three contributions:

$$E_{\mathrm{tot}}(\alpha) = A\int_0^l \kappa^2(s)\,\mathrm{d}s + 2R_0 f_{\mathrm{crit}}\alpha - 2f\Delta y \qquad (4)$$

The first term accounts for the elastic energy of the two bent DNA arms, both of which are identical. The integral goes thus from 0, the end of the chain, to l, the point where the DNA enters the wrapped portion. The prefactor A denotes the bending modulus of the DNA and $\kappa(s)$ its curvature at point s along the contour. The torsional stiffness is neglected because we consider the case of freely-rotating ends, as in the experiment where the DNA is anchored via single-stranded overhangs of the double helix. The second term in Eqn (4) accounts for the wrapped DNA portion. The DNA is assumed to be adsorbed on the protein spool surface along a predefined helical path of radius R_0 with a net adsorption energy density f_{crit}. The degree of DNA adsorption is described by the desorption angle α that is defined to be zero for one full turn of wrapping and to be π for complete unwrapping. Finally, the last term accounts for the external force and is simply given by the product of the applied force f and the DNA end-to-end distance Δy in the force direction.

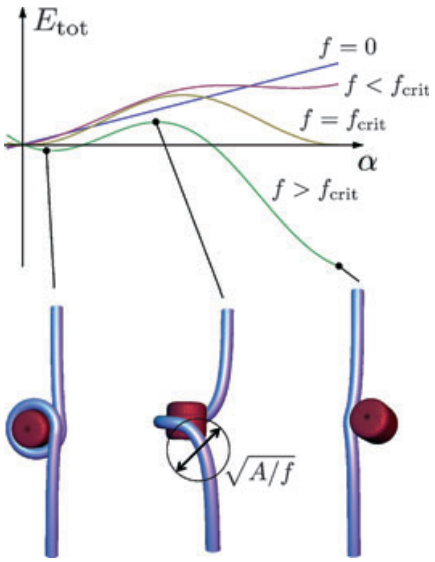

Fig. 2. Model for forced-induced nucleosome unwrapping [32]. Energy landscape for four different cases, $f = 0$, $f < f_{\mathrm{crit}}$, $f = f_{\mathrm{crit}}$ and $f > f_{\mathrm{crit}}$. Example configurations: nucleosome with one wrapped turn (the local minimum), half a turn (transition state) and the unwrapped state (global minimum).

For any value of α, one can minimize the energy of the spool, allowing it to rotate freely to find its optimal orientation. The shape and elastic energy of the DNA arms follows from Euler's 267-year-old theory of elastic rods. In this way, one can compute the energy landscape analytically [32]. Here, we give the most important terms only:

$$E_{\mathrm{tot}}(\alpha) \approx 2R_0(f_{\mathrm{crit}} - f)\alpha - \frac{2}{\sqrt{3}}\sqrt{Af}\cos 2\alpha + \mathrm{const.} \qquad (5)$$

This expression contains a term that tilts the energy landscape and a barrier term. For $f < f_{\mathrm{crit}}$, the global minimum is at the wrapped state; for $f > f_{\mathrm{crit}}$, it is at the unwrapped state (Fig. 2). The barrier in between the two states is proportional to $\sqrt{Af}$. This is a remarkable finding; the experiment that had been set up to determine the barrier actually created it by applying the force!

The f-dependence of the barrier can be explained as follows. To unwrap the last turn, the octamer needs to rotate by 180°. In the transition state (middle example configuration in Fig. 2), the DNA has to make a 90° bend to lead smoothly into the wrapped portion. The length of the bent portion (and its radius of curvature) scales as $\sqrt{A/f}$. The elastic energy is then the ratio of A to that length (i.e. proportional to $\sqrt{Af}$).

First–second round difference

A detailed comparison of the predictions of this model [32] to previous experiments [25] showed that the predicted rates for the passage over the barrier are much too large compared to the rates at which nucleosomes unwrap in the experiment. This forces a critical reconsideration of the assumptions on which the model is based, especially the initially straightforward assumption that the adsorption energy per length is constant along the wrapping path. However, this neglects an important feature of the nucleosome, namely that the two DNA turns interact. Clearly, the turns are sufficiently close to feel a considerable electrostatic repulsion. This suggests that a remaining DNA turn on a halfway unwrapped nucleosome is much more strongly adsorbed then a turn in the presence of the second wrapped turn.

The crucial point is now that the net adsorption energy f_{crit} was estimated from spontaneous unwrapping events of the second turn in the presence of the other turn [8]. f_{crit} might thus have been strongly underestimated because the value $0.3\,k_{\mathrm{B}}T{\cdot}\mathrm{nm}^{-1}$ includes the unfavorable repulsion from the other turn.

To account for this effect, it was suggested that there is a different effective value of f_{crit} for $\alpha > 0$ (i.e. less than one DNA turn) and for $\alpha < 0$ (i.e. more than one turn) [32]. Because the discontinuous unwrapping events observed in the experiment clearly correspond to the case where the last term is being unwrapped (i.e. to the case of $\alpha > 0$), the parameter f_{crit} can be tuned such that one can reproduce the experimental data in a satisfying way. Indeed, a substantially higher effective adsorption energy allows satisfactory fitting of the data [32].

This result might explain how the nucleosome can be accessible to DNA-binding proteins and, at the same time, remain stable. When the nucleosome is fully wrapped, each of the two turns can easily unwrap spontaneously as a result of thermal fluctuations and therefore all DNA is transiently accessible for DNA-binding proteins. What remains puzzling in this set of experiments is why the DNA stops unwrapping further. The interpretation of the unwrapping data suggests that the explanation for this lies in the first–second round difference. Once one turn of the DNA has been peeled off, the remaining turn has a strong grip on the octamer because this turn does not feel the repulsion of the other turn.

Nucleosome sliding

Experimental observation of sliding

We now discuss the sliding of nucleosomes. It has been observed, under well-defined *in vitro* conditions, that nucleosomes are spontaneously repositioned along DNA [33–36]. This mechanism transforms nucleosomal DNA into free DNA and *vice versa*. The repositioning experiments [7] have mostly been performed on short DNA fragments of lengths of $\sim$ 200–400 bp that contain one or two positioning sequences. Repositioning can be detected by gel electrophoresis. One makes use of the fact that the electrophoretic mobility is higher when the nucleosome is located at a DNA end than in the middle of the DNA fragment [33–35]. A different approach [36] makes use of a chemically-modified histone protein that induces a cut in the nucleosomal DNA.

Such studies demonstrated that heat-induced repositioning is typically a slow process, which takes place over a timescale of minutes to hours [33,36] at elevated temperatures (e.g. 37 °C), whereas it is not observed at low temperatures (e.g. 5 °C). Another feature is that the octamer is found at a preferred position (as noted above, the DNA contains a positioning sequence) or multiples of 10 bp (i.e. the DNA helical pitch) apart [33,36]. In addition, there is a preference for end positions [33]. On longer DNA fragments, no evidence for a long-range repositioning has been found [34]. Finally, the presence of linker histones (H1 or H5) that are known to glue the entering and exiting DNA together suppresses nucleosome mobility [35].

Loop defects

What causes nucleosome mobility? It is obvious that an ordinary sliding of the DNA on the protein spool is energetically too costly. As noted above, the interaction between the DNA and the octamer is localized at 14 binding sites. If the nucleosome were to perform a bulk sliding motion, it would have to simultaneously detach from these 14 point contacts to move, for example, by 10 bp. We estimated directly below Eqn (2) that the total binding energy of these 14 sites amounts to $E_{ads} \approx 75\ k_B T$ (this is a lower bound; the first–second round difference suggests an even larger number). Because such a sliding event does not change the bending energy, $E_{elastic}$ (Eqn 1), it is E_{ads} and not E_{net} that has to be overcome. This is, however, too costly to ever occur spontaneously.

There appears to be another possible mode that comes even free of cost. The cylinder could simply roll along the DNA. At one end, it detaches some of the DNA and, at the other, it attaches the same amount, thereby keeping the length of wrapped DNA constant. This simple mechanism, however, does not work. Let us start with a fully-wrapped nucleosome. It is, of course, always possible to detach DNA at one end. However, at the other end, there are no sites where the DNA can bind to because all the binding sites are already occupied. Continuing the rolling motion in one direction, the octamer would simply roll off the DNA. This mechanism could only work if the octamer comprised an infinitely long cylinder with an infinitely long helical binding path.

Repositioning must thus involve intermediates with a lower energetic penalty. The two most likely candidates [7,37] are based on small defects that spontaneously form in the wrapped DNA portion and propagate through the nucleosome: 10 bp bulges [38,39] (Fig. 3A) and 1 bp twist defects [40] (Fig. 3B).

The basic idea of the bulge mechanism is as follows: first, some of the DNA spontaneously unwraps from one of the termini of the wrapped portion [8,20], as discussed above with respect to nucleosome breathing. In most cases, the DNA readsorbs back to the fully wrapped state from which it started. From time to time, however, the DNA is pulled in and then readsorbs. This creates an intranucleosomal DNA bulge

that stores some extra length ΔL. This bulge diffuses along the wrapped DNA portion and finally leaves the nucleosome at either end. If the loop comes out at the end where it was formed, the nucleosome is back at the original state. However, if the loop leaves at the other end, the stored length ΔL has effectively been transported through the nucleosome and the nucleosome has moved by that amount along the DNA.

A careful quantitative analysis, again based on the theory of Euler's elastica, has been provided [39]. It was found that the cheapest small loop has a length of $\Delta L = 10$ bp (Fig. 3A). Other loops are far more expensive because they require twisting and/or stronger bending. However, even a 10 bp loop is very expensive because its formation requires $\sim 20\, k_B T$ of desorption and bending energy. As a consequence, the corresponding diffusion constant of the octamer along the DNA was found to be very small, namely on the order of $D \approx 0.1$ bp^2·s^{-1}. This leads to typical repositioning times on a 200 bp DNA fragment that are on the order of a few hours, which is in reasonable agreement with the experimental data [33,36]. The strong temperature dependence and, most strikingly, the preference for 10 bp steps (i.e. corresponding to the extra length stored in the cheapest loops) are also in reasonable agreement with the experiments. All these facts strongly indicate that the loops comprise the mechanism underlying the repositioning. Nevertheless, as we discuss below, twist defects also lead to predictions that are consistent with these experimental observations.

Twist defects

The basic idea of the twist defect mechanism is similar to that of the bulge mechanism. Here, a twist defect forms spontaneously at either end of the wrapped DNA portion. Such a defect carries either a missing or an extra base pair. A defect with a missing base pair is shown in Fig. 3B. A defect is localized between two neighboring nucleosomal binding sites (i.e. within one helical pitch, 10 bp). This short DNA portion is stretched or compressed and, at the same time, over- or undertwisted. The energy of a $\pm$ 1 bp twist defect was estimated from the stretch and twist elasticity of DNA to be on the order of 10 $k_B T$ [40]. This means that, at a given time, one finds a twist defect only on one out of $\sim$ 1000 nucleosomes.

Once a twist defect has formed, it diffuses through the wrapped DNA portion. The nucleosome provides, between its 14 binding sites, 13 positions for the defect. A defect (e.g. say a 'hole' with a missing base pair) moves from one position to the next in the fashion of an earthworm creeping motion. This is depicted in Fig. 4, where we represent the DNA by a bead-spring chain, with the beads corresponding to the base pairs. Also indicated are the binding sites, which are shown for simplicity at an equilibrium distance of three instead of 10 beads. The base pair that is in contact with a binding site moves towards the defect, resulting in an intermediate state where the defect is stretched out over 20 bp, which lowers the elastic strain but has costs relating to desorption energy; the latter is rather expensive, 12 $k_B T$, taking the first–second round difference into account. Once the next base pair has bound to the nucleosome, the twist defect has moved to the

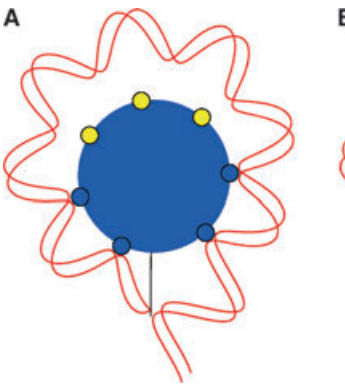

Fig. 3. Defects on the nucleosome that might underlie nucleosome sliding. (A) Loop defect. (B) Twist defect.

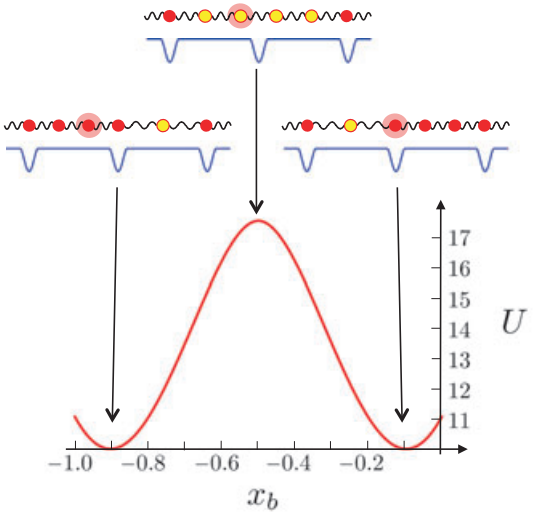

Fig. 4. Energy landscape felt when a twist defect moves from one location to the next. The energy cost U in units of $k_B T$ for a defect as a function of the position of the marked bead. Example configurations are also shown. For simplicity, we depict here a distance of three beads (=bp) between two binding sites.

neighboring location. At the beginning, the bead that eventually will be bound is at position $x = -0.9$ (in dimensions of the base pair step length) and the energy is 10 k_BT compared to the defect-free nucleosome. At the end, this bead is bound, $x = -0.1$, and the energy is again 10 k_BT. In between is a barrier of height, $\Delta U \approx 7.5\ k_BT$, which is reached at $x = 1/2$.

Not all twist defects that have formed reach the other end of the nucleosome; most fall off at the end where they have been created. Assuming that all 13 possible defect locations are energetically equivalent, one can show that only one of 13 of the defects ultimately reaches the other end. Every successful twist defect causes a 1 bp step and a rotation by 36° around the DNA axis. As a result, the DNA performs a corresponding corkscrew motion on the nucleosome. Taking everything into consideration (i.e. the barrier crossing of defects, the probability for defects to be successful and an elementary hydrodynamics argument), one can predict the diffusion constant of the nucleosome along the DNA to be on the order of 70 $bp^2 \cdot s^{-1}$. With this result, one can predict redistribution times on a 200 bp segment in the range of seconds, which is far too fast compared to experimental observations. Even worse, there is no 'built-in' mechanism for 10 bp steps of the octamer along the DNA. The experimentally observed preference for positions 10 bp apart, manifesting itself in characteristic bands in the products of gel electrophoresis [33,34], initially appears to be inconsistent with this mechanism.

An important additional feature of the repositioning experiments now comes into play, namely that they are typically performed with DNA segments containing strong positioning sequences, especially the sea urchin 5S positioning element [33–35]. Such sequences show highly anisotropic bendabilities of the DNA. If repositioning is based on twist defects, then the DNA has to bend in the course of a 10 bp shift in all directions, and thus has to go over a substantial barrier. The elastic energy of the bent DNA is then an approximate periodic function of the nucleosome position, with the helical pitch being the period. This energy was described previously [40] by an idealized potential of the form $U(l) = (A/2)\cos(2\pi s/10 + \varphi)$, with l being the base pair number; A denoting the difference in elastic energy between the optimal and the worst rotational setting; and φ representing some phase factor. In principle, these oscillations die out completely when the nucleosome leaves the positioning sequence (i.e. if it has moved ~ 140 bp). However, because the templates are usually quite short (e.g. 207 bp) [33], the nucleosome always feels the rotational signal from the positioning sequence and such a periodic elastic energy should provide a reasonable description. For $A > k_BT$, the nucleosomal diffusion constant is reduced to [40]:

$$D \approx D_0 \frac{\pi A}{k_B T} e^{-A/k_B T} \quad (6)$$

where D_0 denotes the diffusion constant for homogeneously bendable DNA, $D_0 \approx 70\ bp^2 \cdot s^{-1}$.

For the sea urchin 5S positioning element, one has $A \approx 5\ k_BT$ based on the analysis of dinucleotide frequencies as a function of the position inside a nucleosome as found in various studies [41]. According to Eqn (6), this reduces the diffusion constant by approximately one-tenth to $D \approx 7\ bp^2 \cdot s^{-1}$. This is close to the experimental value, even though it might still overestimate the observed value. Moreover, it also explains the preference for certain positions with a 10 bp spacing: this just reflects the Boltzmann weight, where it is $e^{10} \approx 10^4$ times more likely to find the nucleosome at a favorable rotational setting compared to the most unfavorable one.

Loop versus twist defects

From the above considerations, it becomes clear that both mechanisms (i.e. the 10 bp loop and the 1 bp twist defect) lead to much the same predictions in the presence of rotational positioning sequences. Given the uncertainties of the precise values of the barriers that the defects have to cross, both mechanisms are possible candidates for the experimentally observed repositioning, even though the elementary motion is fundamentally different. This raises the question of whether there are additional experimental observations that could distinguish between the two cases.

The most straightforward experimental test would be to study repositioning on DNA fragments that do not contain positioning signals. If repositioning is based on twist defects, one should observe a dramatic increase in nucleosomal mobility. The experiments are geared, however, towards using positioning sequences. They help the loading of nucleosomes onto DNA and provide clear signals in the form of bands observed on gel electrophoresis. Positioning signals also help to compete with the preference of nucleosomes to sit at DNA ends.

One exception is provided by work on nucleosome sliding on telomeric DNA [42–46]. Telomeric DNA sequences feature short repeat sequences, typically 6–8 bp in length, that are not commensurate with the DNA helical pitch. Such a DNA chain can thus not feature strong positioning signals. Indeed, it was found that nucleosomes reposition substantially faster on telomeric DNA than on DNA featuring an average

sequence [45]. This strongly indicates that nucleosome mobility is caused by twist defects.

A further experiment on DNA including a positioning sequence supports the twist diffusion picture. Gottesfeld *et al*. [47] studied repositioning on a 216 bp DNA fragment that contained the sea urchin 5S rDNA nucleosome positioning sequence. The experiment was performed in the presence of pyrrole-imidazole polyamides, synthetic minor-groove binding DNA ligands that are designed to bind to specific target sequences. After 1 h of incubation at 37 °C in the absence of any ligand, redistribution of the nucleosomes was observed. This redistribution was completely suppressed in the presence of 100 nM ligands if the target sequence of this specific ligand faced outside (i.e. towards the solution) when the nucleosomal DNA was bent in its preferred direction. On the other hand, a ligand whose binding site faced the octamer in its preferred rotational frame had no detectable effect on the repositioning dynamics. As a result of the ligands binding into the minor groove [48], it is quite likely that a bound ligand will block the overall corkscrew motion of the DNA because the DNA can only rotate on the nucleosome up to a point where the bound ligand comes close to one of the 14 binding sites. Such a steric effect should not be expected to occur if repositioning is based on bulges. A quantitative theoretical analysis of this experiment [49] provided additional support for the twist diffusion picture.

Another interesting approach is to apply a small force on the nucleosome to induce its sliding along the DNA chain. One should then expect that the nucleosome movement is induced by the same type of defect as for the case where thermal effects alone cause repositioning. A possible experimental set-up is to have an RNA polymerase transcribing along DNA and then to study what happens when such a polymerase encounters a nucleosome. This should be also a common event in a cell because tens to hundreds of nucleosomes are engaged with a given gene. A series of experiments [50–53] reports that RNA polymerase transcribes through nucleosomes. These are, however, highly artificial *in vitro* set-ups using bacteriophage RNA polymerase (which never encounter nucleosomes in real life) performed on short DNA templates (e.g. 227 bp) [50] containing one nucleosome. The unexpected finding of this set of experiments was that the polymerase can transcribe through the nucleosome and that, as a product of this reaction, one has a full-length transcript and the nucleosome at a new upstream position on the DNA template. Although a similar finding has also been reported for eukaryotic RNA polymerase III [54], RNA polymerase II typically causes the loss of an H2A/H2B dimer, whereas nucleosome repositioning is not detectable [55].

To explain this remarkable result, Studitsky *et al*. [50] proposed that the polymerase could cross the nucleosome in a loop that might particularly explain why the nucleosomes move upstream. However, this model is not consistent with the findings of the study by Gottesfeld *et al*. [47], who found that the polymerase becomes stuck once it encounters nucleosomes whose mobility is suppressed as a result of the presence of a minor-groove binding ligand. An alternative explanation, which is also consistent with the latter observation, is that the polymerase pushes the nucleosome in front of it, inducing small twist defects that mobilize the nucleosome. The force required to induce this motion can easily be provided by the polymerase [56]. The upstream movement of the nucleosome might not then be caused by a loop. Instead, the octamer is not completely pushed off the DNA template and the other free end (i.e. the one upstream) of the DNA recaptures the octamer. As a result, the octamer is effectively transferred to an upstream position [49].

A recent experimental study [57] of the transcription of RNA polymerase II through a nucleosome indicates that the polymerase transfers the nucleosome in a loop. In that study, the polymerase and the upstream end of the DNA were attached to optical beads, allowing control of the tension. At the other end of the DNA (i.e. downstream), a nucleosome was bound to the high-affinity 601 nucleosome positioning sequence. The experimental observations suggested that the nucleosomes were transferred upstream when transcription was performed in the absence of an external force, although they were mostly lost when forces of a few pN were applied. This supports the idea that the polymerase crossed the nucleosome in a loop as long a loop formation was not too costly (i.e. at sufficiently small forces).

This finding might point toward an interesting possibility. When positioning is too strong, then nucleosome sliding through twist defects is prohibitively expensive. In this case, nucleosomes do not slide at all, or very rarely and then through a loop defect. A nucleosome in a super strong positioning sequence (such as 601) is pinned to that position and can only be moved out of it through an active mechanism such as the one previously observed [57].

Activated nucleosome dynamics

In the previous sections of this minireview, we have analyzed the response of nucleosomes to thermal energy and to the effects of external forces. In this

final section, we move onto the activation of nucleosome dynamics by enzymatic processes involving the consumption of ATP. We begin with a brief description of the families of enzymes involved in these processes, the chromatin remodelers [58–60], but refer also to the minireviews of our colleagues who provide more detail on these families [61] and on the single-molecule experiments on chromatin remodelers [62].

A further important feature of this discussion involves the role of the histone tails and the modifications that they carry; the histone tails have played no part in the discussion so far, although, subsequently, they will get a prominent role.

Chromatin remodelers and histone tail modifications

In a follow-up investigation to their study of the 'site exposure' model, Polach and Widom [8] looked at the effect of histone tails [63], mostly aiming to determine whether the presence of the tails might affect the use of tailless nucleosomes as a model system. The results obtained in their study showed that the removal of tails leads to a small, but significant (1.5- to 14-fold) increase in the position-dependent equilibrium constants for site exposure. The tails also contribute, although only modestly, to the positioning-specificity of the nucleosomes. In particular, Polach and Widom [8] reason that acetylated tails will not significantly affect accessibility, so that their presence at this level does not constitute a prerequisite for transcription activation.

These results should be seen in the context of chromatin remodeling comprising an activated process; equilibrium accessibility is not all that counts. Although acetylation of the tail will bring a nonspecific background effect as a result of the difference in electrostatic charge that they cause between the tail and the DNA, a more specific interaction might indeed be of much more importance.

Chromatin remodelers use the energy provided by ATP to act on nucleosomes. Consequently, all remodelers contain an ATPase subunit that is derived from the SWI2/SNF2-family. In these enzymes, the ATPase domain is split in two parts: DExx and HELICc. Although the ATPase subunit is that of a helicase, remodelers have so far not shown helicase activity. The distinction between the different remodelers arises mainly from the domain neighboring the ATPase domains that decides on the functionality of the enzymes. These differences allow us to distinguish between four families: SWI/SNF, ISWI, CHD and INO80 [59].

Focusing on the SWI/SNF and ISWI, one can in particular identify a bromodomain in the C-terminal region of SWI/SNF, and the SANT and SLIDE domains in ISWI. Bromodomains interact with acetylated lysines and are capable of recognizing correspondingly modified histone tails [64]. Notably, the bromodomains can also arise in multiple copies in the different variants of the remodelers. By contrast, the SANT domain of the ISWI remodelers is known to interact with unmodified histone tails.

One therefore has two distinguished cases in which specific histone tail modifications are read by two different members of the remodeler families. Indeed, both these remodelers are implied in antagonistic mechanisms: SWI/SNF is involved in transcriptional activation, whereas ISWI plays a role in repression because its function is to arrange the nucleosomes in a regular way, helping the condensation of chromatin.

A special role for ATP: kinetic proofreading

The above discussion shows that there are three levels on which the biological function, the biophysical (mechanical) action of the remodelers, and the biochemical modification of the histone tails are related:

transcriptional activation ↔ SWI/SNF ↔ acetylation
and
transcriptional repression ↔ ISWI ↔ deacetylation

Recently, we have postulated a biophysical model that can explain the logic behind this triad [65]. We have shown that, in the process of reading-out (e.g. the specific acetylation on the histone tail), the recruitment step of the recognition unit of the remodeler (because it is directly coupled to the ATPase) allows the system to undergo an ATP-dependent remodeling step. The coupling of a discriminating recognition step (i.e. here between an acetylated and a non-acetylated histone tail) to a step consuming ATP is the hallmark of a kinetic proofreading scenario (Fig. 5) [66]. The recognition step is quantitatively determined by the free energy, ΔG, of complex formation. Because of the kinetic proofreading mechanism, this free energy is essentially doubled: $\Delta G \rightarrow 2\Delta G$. Because the free energy enters a Boltzmann factor $\sim \exp(-2\Delta G/k_{\mathrm{B}}T)$, the recognition of the 'correct' substrate is therefore significantly favored by the involvement of the remodeler.

Is there experimental evidence for such a scenario? We discuss two cases: the activation of the gene for INF-β, a possible test case for the transcription initiation scenario, and the case of ISWI-remodeling, for

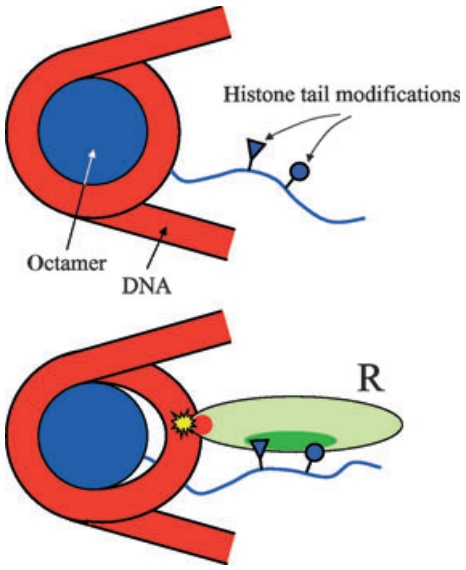

Fig. 5. Sequence of regulatory events assumed in the kinetic proofreading model. Top: nucleosome with a histone tail carrying amino acid modifications. Bottom: recruitment of a remodeling complex *R* with a histone modifier recognition domain (dark green) and an ATP-domain (red). The DNA is partially loosened from the histone octamer. Additional recruitments of transcription factors are omitted. The size of the nucleosome and the remodeler are not shown to scale: the overall remodeler size, all domains confounded, is larger than that of the nucleosome.

which a recent proposal for a kinetic proofreading scheme in the context of transcriptional repression has been made [67].

The gene for INF-β is activated in response to a viral infection. It has been studied in HeLa cells after infection with Sendai virus in a series of experiments by Agalioti *et al*. [68,69]. In particular, it was possible to disentangle the sequence of events leading to the activation of the gene [69]. After the assembly of the enhanceosome upstream of the gene, recruitment of the acetylating enzyme gcn5 occurs, which acetylates lysine residues on the histone tails: first, H4K8 and H3K9, and then H3K14, after an intermediate phosphorylation step.

The tail mark H4K8 is specifically read by the bromodomain of the SWI/SNF (BRG1) remodeling complex. Removal of the H4-tail suppresses recruitment, and changes in the modification patterns impede the recognition. Preliminary protein–peptide docking calculations on the specificity of the formation of histone tail bromodomain complexes support this finding in that specific modifications or combinations of modifications are observed to have significantly different energies (J.C. Boisson, F. Cleri & R. Blossey, unpublished data), which is an essential condition for the kinetic proofreading scenario outlined above.

We finally turn to the ISWI remodeler system, which has been analyzed in detail over the last few years [71–73], and whose properties in mammalian cells are reviewed in this minireview series by Erdel and Rippe [74]. IWSI remodelers are involved in the generation of repressed states of chromatin by moving the nucleosomes into ordered arrays, favoring their condensation into repressed chromatin. The deciphered mechanisms are very rich because they involve the action of ISWI remodelers as dimers. Furthermore, they require that the remodelers detect both DNA length and histone tails. Focussing on the role of the H4 tail, the latter has been found to play a decisive role in ISWI remodeling: not only both the complete removal of the H4 tail [75–77], but also its site-specific acetylation [78] suppressed the remodeling action of ISWI. These findings are therefore similar to that of the gene for INF-β, offering interesting avenues for the demonstration that kinetic proofreading scenarios may be relevant to remodeling.

Conclusions

In this minireview, we have provided an overview on the dynamics of nucleosomes under the influence of thermal energy, applied forces and ATP-dependent remodeling. Concerning the thermal activation of nucleosomes, we have discussed breathing, as well as the diffusion mechanisms, from a theoretical perspective, building on experimental evidence. In our view, a substantial understanding of this problem has been reached, although it is clearly not yet fully resolved.

As far as the ATP-dependent processes of chromatin remodeling are concerned, the field is clearly still at its beginning. Experimental and theoretical studies are needed to understand how nucleosomes are actively moved and how the underlying regulation works. Kinetic proofreading appears to represent an interesting scenario because it opens up an exciting possibility of the regulated control of chromatin structure beyond cooperative mechanisms. It remains to be seen how far such scenarios can be demonstrated experimentally, and how general their role is in modulating chromatin structure and gene regulation.

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