CHROMATIN BIOLOGY

Sliding nucleosomes with a twist

DNA twist, driven by ATPases, ratchets DNA past the histone core

By **Gregory D Bowman**[1] *and* **Sebastian Deindl**[2]

As the fundamental packaging unit of eukaryotic chromosomes, nucleosomes restrict accessibility of DNA. Nucleosome placement along DNA can therefore dictate how the genome is read out by cellular machinery, including whether particular genes are turned on or off. Placement and repositioning of nucleosomes throughout the genome is achieved by ATP-dependent chromatin remodelers (remodelers), which use a common ATPase motor to disrupt and reorganize DNA packaging. At long last, recent structural, biophysical and biochemical work has revealed a core mechanistic framework for nucleosome sliding, where remodelers capitalize on distinct energetic and structural states of duplex DNA as it twists and bends around the nucleosome.

Nucleosomes are the most ubiquitous protein-DNA complexes in all eukaryotic cells. Consisting of ~146 base pairs (bp) of duplex DNA spooled around a cylindrical histone core (*1*), nucleosomes are repositioned when DNA shifts past the histone core, superficially like a rope being pulled past a pulley. By virtue of their extensive protein-DNA interface, nucleosomes are relatively stable structures. A major challenge has been understanding how such a stable interface is dynamically disrupted and re-established during ATP-dependent nucleosome sliding.

Remodelers are the protein machines charged with altering the position, occupancy, and histone composition of nucleosomes. Grouped into distinct families, remodelers display a wide range of subunit composition and include large, multi-subunit mega-Dalton complexes (such as SWR1, INO80, and SWI/SNF) as well as smaller complexes (ISWI) and exclusively monomeric (Chd1) enzymes. Whereas each remodeler type has family-specific subunits and protein domains, consistent with distinct biological roles, all share the ability to manipulate nucleosome structure. This ability stems from the one element common to all remodelers - a highly conserved ATPase motor that belongs to a larger superfamily of helicase-like ATPases called superfamily 2 (SF2). Many SF2 enzymes can translocate along DNA or RNA by walking along a single strand, which is termed the tracking strand. Based on years of intense biophysical and biochemical work on SF2 and the related SF1 ATPases, insights on how these enzymes move and interact with nucleic acids have set the stage for understanding how remodeler ATPases move and engage DNA (*2*).

Both SF1 and SF2 ATPases consist of two distinct RecA-type lobes that together form an ATP-binding pocket and a nucleic acid binding surface across the central cleft. Dictated by the occupancy of the nucleotide-binding pocket, the two lobes open and close like a clamshell, which in turn alters interactions with the bound nucleic acid. For DNA translocases, the two lobes are splayed apart in an open conformation in the nucleotide-free state, both interacting with the tracking strand. Upon ATP binding, the two lobes favor a closed conformation, with the first lobe slipping along DNA by one nucleotide (nt). Following ATP hydrolysis, they revert to an open conformation, allowing the second lobe to reach ahead on the DNA by one nt. This ATP-driven cycle of opening and closing thereby allows the ATPase to translocate along DNA in what has been dubbed an inchworm-type motion (*2*).

Consistent with an inchworm-like translocation mechanism, nucleosomal DNA is shifted by remodelers with an elementary step of a single bp (*3*). As such, nucleosomal DNA must therefore shift around the histone core in a corkscrew fashion. Remodeler ATPases remain in a fixed location on the histone core during DNA translocation, which means that DNA all around the nucleosome must shift in response to localized action at the ATPase binding site. According to the classic interpretation of the inchworm model, DNA on both sides of the ATPase should be shifted simultaneously with each hydrolysis cycle, requiring that the motor be physically coupled to the histone core to generate force. Instead, remodeler ATPases shift nucleosomal DNA discontinuously, with DNA movement resulting from both open and closed states of the ATPase that bypass the strict requirement for a separate histone foothold to push against (*4*). In its open (nucleotide-free and ADP-bound) state, the ATPase pulls entry side DNA toward itself in a corkscrew fashion, creating a small DNA bulge at the binding site. When poised for hydrolysis, the ATPase in its closed state eliminates this bulge, corkscrewing DNA toward the dyad on the other side (Fig. 1). This creation and elimination of a DNA bulge is equivalent to altering DNA twist.

Changes in DNA twist, known as twist defects, have been proposed as a low-energy means of ratcheting DNA past the histone core (*5*). Twist defects form at the junction between two segments of DNA, one that undergoes a corkscrew shift (mobile) and another that remains stationary with respect to the histone core. Twist defects therefore reflect a gain or loss of a bp resulting from the corkscrew shift of DNA. Twist defects dissipate once one side of the junction undergoes a compensatory corkscrew shift, restoring DNA to its canonical conformation on the nucleosome. If the corkscrew shift occurs on the previously stationary DNA segment, another twist defect can be created further downstream where the mobile DNA segment again runs up against a stationary segment. Through such discontinuous motions, propagation of twist defects all the way around the nucleosome repositions the entire length of nucleosomal DNA relative to the histone core. Spontaneous nucleosome sliding via twist defects, recently visualized through molecular simulations, suggested that twist can be absorbed or buffered at a site on the nucleosome called superhelix location 2 (SHL2) (*6*), in agreement with variations in DNA twist previously observed in nucleosome crystal structures (*1*).

Experimental support for twist diffusion occurring over multiple catalytic cycles of a remodeler ATPase was recently provided by 3-color single-molecule FRET (*7*). As observed using FRET reporters on both sides of the nucleosome, DNA on the entry side shifted onto the nucleosome before DNA on the exit side was shifted off. Importantly, the delay between movement of entry and exit DNA was ATP-dependent, with lower ATP concentrations yielding longer delays between entry and exit DNA movements. The

[1]*T.C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, USA.* [2]*Department of Cell and Molecular Biology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden. Email: gdbowman@jhu.edu; sebastian.deindl@icm.uu.se*

ATP-dependent delay means that even after going through a full ATP hydrolysis cycle to pull DNA onto the entry side, the remodeler requires one or more ATP-binding and hydrolysis events to push DNA out the exit. During this delay, the nucleosome absorbs one or more bp of DNA pulled on by the remodeler, which would take the form of twist defects. An intriguing question for future studies is whether such DNA buffering and changes in twist enable communication between pairs of remodelers on opposite sides of the nucleosome (*7*).

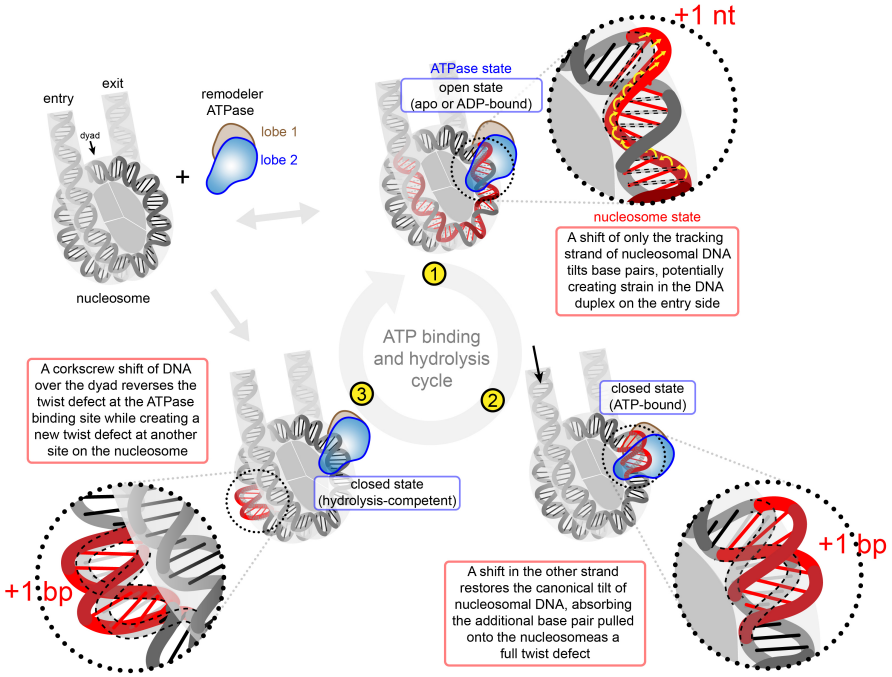

Figure 1. A model of how twist defects, stimulated by the remodeler ATPase, shift DNA around nucleosome. Twist defects are not expected to form when the ATPase binds in the ATP-bound state (step 3). After a full cycle (steps 1→2→3), creation and passage of additional twist defects from subsequent cycles push DNA out the exit side.

Recently, key stages in the nucleosome sliding cycle have been captured by cryo-EM, adding essential mechanistic insight into how repositioning is achieved. In the initial stage, the open state of the ATPase bound at SHL2 remarkably creates a bulge in only the tracking strand of the DNA duplex, but not the complementary guide strand (*8*) (Fig. 1, step 1). A consequence of pulling only one of the two DNA strands is base tilting, necessary to maintain base pairing between strands. Interestingly, a similar tilting of base pairs was observed in the DNA-RNA hybrid cradled by RNA polymerase (*9*, *10*), and may create strain in the duplex. For remodelers, strain from bp tilting would prime the duplex for the next stage, when a compensatory shift of the other strand creates a full twist defect (step 2). An example of a full twist defect with both DNA strands bulging by 1 bp at SHL2 has been observed in the SWR1-nucleosome complex (*11*). In the final stage, after remodelers have successfully shifted the twist defect toward the dyad, the DNA at SHL2 should return to its canonical structure on the nucleosome (step 3). Such a post-shifted state with canonical DNA twist at SHL2 has been visualized in remodeler-nucleosome complexes with the ATPase bound to ADP·BeF_3^- (*8*, *12*, *13*). In addition to ADP·BeF_3^-, canonical DNA twist is also favored by the ATPase bound to the transition state mimics ADP·AlF_X and ADP·MgF_X (*4*). Since nucleosomal DNA with a canonical twist aligns with the transition state of the ATPase, elimination of the twist defect may be sufficient to trigger ATP hydrolysis, which would initiate a new round of sliding and enforce directionality of twist diffusion.

With the core mechanism of nucleosome sliding finally coming into focus, this recently developed framework provides a launching point for exploring a host of new exciting questions. In addition to DNA geometry and energetics, to what extent is twist diffusion dependent on other characteristics of the nucleosome? The histone core has been shown to have dynamic properties (*14*, *15*), and an important goal is determining the degree that plasticity of histone structure may impact formation and propagation of twist defects within the nucleosome. Histone proteins come in a variety of flavors, and it will be of great interest to identify whether distinct biophysical properties of histone variants can determine or bias the outcomes of remodeling reactions.

The ability of remodeler-type ATPases to create twist defects is used for more than just sliding nucleosomes. The SWR1 remodeler, for instance, does not reposition the histone core but instead is specialized for swapping out canonical histone H2A/H2B dimers for variant H2A.Z/H2B dimers. A key issue for this system will be uncovering the connection between twist defects and dimer exchange. More strikingly, remodeler-type ATPases also include enzymes that act on non-nucleosomal substrates: Mot1 (TBP/DNA), Rad54 (Rad51/DNA), the bacterial RapA (RNA polymerase/DNA), and CBP (RNA polymerase/DNA as well as nucleosomes), with each disrupting otherwise stable protein-DNA complexes. As remodeler-type ATPases, they likely act by distorting duplex DNA, creating high-energy intermediates analogous to twist defects on the nucleosome. Just as insights for SF1 and SF2 ATPases have facilitated thinking about nucleosome sliding, the discovery that remodelers create and eliminate twist defects on the nucleosome will likely advance understanding for these related yet functionally distinct ATPases, which all wrestle the double helix to transiently blaze a trail through the energetic landscape of duplex DNA.

REFERENCES

1. K. Luger et al., *Nature* **389**, 251 (1997).
2. M. R. Singleton *et al.*, *Annu. Rev. Biochem.* **76**, 23 (2007).
3. S. Deindl *et al.*, *Cell* **152**, 442 (2013).
4. J. Winger *et al.*, Elife **7** (2018).
5. K. E. van Holde *et al.*, *Structure and Function of the Genetic Apparatus*, 35 (1985).
6. G. B. Brandani *et al.*, *Nucleic Acids Res.* **46**, 2788 (2018).
7. A. Sabantsev *et al.*, *Nat. Commun.* **10**, 1720 (2019).
8. M. Li et al., *Nature* **567**, 409 (2019).
9. A. L. Gnatt *et al.*, *Science* **292**, 1876 (2001).
10. A. C. M. Cheung *et al.*, *Nature* **471**, 249 (2011).
11. O. Willhoft *et al.*, *Science* **362** (2018).
12. L. Farnung *et al.*, *Nature* **550**, 539 (2017).
13. R. Sundaramoorthy *et al.*, *Elife* **7** (2018).
14. K. K. Sinha *et al.*, *Science* **355** (2017)
15. S. Bilokapic *et al.*, *Nat. Commun.* 9, 1330 (2018).

We apologize for work that could not be cited due to space constraints.

ACKNOWLEDGMENTS

We thank Ilana Nodelman and Anton Sabantsev for discussions and critical comments. G.D.B. is supported by the NIH (R01-GM084192). S.D. acknowledges support from ERC (ChromatinRemodelling), VR, and the KAW foundation.