Published in final edited form as:

J Biophotonics. 2008 September ; 1(4): 280–286. doi:10.1002/jbio.200810027.

ATP-dependent looping of DNA by ISWI

Giuseppe Lia¹, Marco Indrieri⁴, Tom Owen-Hughes², Laura Finzi³, Alessandro Podesta⁴, Paolo Milani⁴, and David Dunlap^{5,*}

¹Harvard University, Chemistry & Biological Chemistry Dept., Cambridge, MA, USA ²Division of Gene Regulation and Expression, The Wellcome Trust Biocentre, Department of Biochemistry, University of Dundee, Dundee Scotland ³Department of Physics, Emory University, Atlanta, GA, USA ⁴Department of Physics, University of Milan, Milan Italy ⁵Department of Cell Biology, Emory University, Atlanta, GA, USA

Abstract

Alteration of chromatin structure is key in the regulation of gene transcription. Some protein complexes remodel chromatin in an ATP-dependent manner to favor access to particular sequences. These chromatin remodeling factors form four families, whose archetypes are the yeast RSC (SWI/SNF) complex, the fly ISWI, the mouse CHD1 and the yeast INO80. All possess an ATPase subunit similar to the SF-II helicases which hydrolyze ATP to track along DNA. Translocation and the resulting torque in the DNA could drive chromatin remodeling. While the RSC complex exhibits ATP-dependent translocation and introduces negative supercoils into bare DNA, the ISWI complex was believed to be inactive on bare DNA. However new tethered particle motion assays and AFM images show that in absence of ATP, ISWI binds the DNA molecule wrapping it in an histone-like manner. In the presence of ATP, ISWI generated loops with negative supercoils.

Introduction

Chromatin remodelling proteins are a burgeoning group of ATP-hydrolyzing machines that are critical for embryogenesis and development ¹. These proteins direct access to DNA by regulating chromatin structure on several levels ². Their trademark biochemical functions are the mobilization or modification of nucleosomes to expose appropriate DNA sequences. These proteins have been grouped into four functionally/structurally similar families: SWI/SNF, ISWI, NURD/Mi-2/CHD, and INO80 and SWR1 ³. The SWI/SNF and ISWI families have been best characterized and although they both utilize ATP to power DNA translocation, they remodel chromatin in distinct ways. While ISWI repositions nucleosomes to create ordered arrays ⁴, SWI/SNF repositions nucleosomes and catalyzes histone subunit exchange and modification ⁵. A number of SWI/SNF-type chromatin remodelling proteins have been well characterized using single molecule assays. For example the RSC protein was shown to translocate DNA at up to 200 base pairs/s to create a negatively supercoiled loops 450 base pairs (bp) long ⁶. Similar translocation on nucleosomal substrates slowed to 13 bp/s for 100 bp distances but only stalled for forces above 12 pN ⁷. RSC is a large complex and the structure of the yeast homolog includes a large cavity in which extensive contact with the nucleosomal DNA most likely identifies the translocase domain ^{8,9}.

Although equivalent structural detail has yet to become available for ISWI family members, careful footprinting assays have shown regions of contact with nucleosomes to suggest a

*To whom correspondence should be addressed .

pumping mechanism that drives a small loop of DNA around the histone octamer ¹⁰. This data and photochemical crosslinking of ISW2 ¹¹ indicate a translocase domain positioned near the dyad of the histone octamer with other domains that span the nucleosome to contact extranucleosomal DNA. Earlier biochemical studies have shown that ISWI family ATPases can translocate on nucleosomal substrates ¹² and generate superhelical tension ¹³. That data revealed that DNA templates can stimulate the ATPase activity of ISWI although nucleosomal substrates were required for the generation of superhelical tension. Work on the ISW2 protein also showed complex formation with DNA templates ¹⁴. Complexes formed with or without ATP but hydrolysis to ADP disrupted the interaction. To extend these studies, atomic force microscopy (AFM) and tethered particle motion (TPM) experiments were performed using recombinant *Drosophila melanogaster* ISWI with DNA templates. DNA was observed to wrap around the protein and ATP drove the formation of supercoiled loops.

Experimental

Preparation of the flow chamber for tethered particle motion

The flow chambers for TPM measurements were similar to those described by Finzi and Dunlap ¹⁵. A flow chamber was incubated for 30 minutes with 20 µg/ml anti-digoxigenin (Sigma, St. Louis, MO) in PBS. After washing the flow chamber with 800 µl of ISWI buffer (50 mM KCl, 10 mM HEPES (pH 7.8), 3 mM MgCl₂, 0.1 mM DTT and 60 µM BSA) a 900 bp biotin- and digoxigenin-labelled DNA amplified from pUC19 was introduced and incubated for 1 hour before washing out unbound DNA with 800 µl ISWI buffer. An excess of 430 nm diameter, streptavidin-coated beads (Indicia, Oullins, France) in ISWI buffer was then introduced and incubated for 30 minutes. Unattached beads were then flushed from the chamber with ISWI buffer. Experiments were carried out in ISWI buffer and 20 nM concentrations of protein. Recombinant *Drosophila* ISWI was purified as described previously ¹⁶.

Tethered particle motion (TPM) measurement

Video-enhanced, differential interference contrast micrographs of the tethered beads were recorded with a CCD camera (JAI A60, Copenhagen, Denmark) at 25 frames/s on a SuperVHS recorder. Images were digitized with a PCI-1409 frame grabber (National Instruments, Austin, Texas, USA) and analyzed using custom software to determine the x and y coordinates of the bead versus time. The mean of the x and y coordinates establish the anchor point of the DNA on the glass. The Brownian motion of the bead was then calculated

as $R = \sqrt{(x - \bar{x})^2 + (y - \bar{y})^2}$ and smoothed with a 100-point, moving average.

AFM imaging

DNA was synthesized by PCR amplification of an 891bp segment of the plasmid pUC19 and purified with a PCR purification kit (Qiagen, Hilden, Germany). About 100 ng of this DNA was incubated for 8 min with equimolar amounts of ISWI complex in ISWI buffer (without BSA and with or without ATP(25µM)). After incubation, 5-10 µl were deposited at room temperature for 2-3 min on freshly cleaved mica (Ted Pella Inc., Redding, CA) coated with poly-L-ornithine (MW 35,000 Sigma). The mica was then gently washed with 1ml of HPLC grade H₂O (Sigma) and subsequently dried with a stream of nitrogen gas. The sample was imaged in air with a NanoScope III (Digital Instruments, Santa Barbara, CA) atomic force microscope operating in tapping mode. The AFM images were analyzed with the open source program ImageJ (NIH, Bethesda, MD) to determine the DNA contour length and

with the program WSxM (Nanotech, Inc., Madrid, Spain) to determine the height and diameter of the complex.

Supercoiled plasmid analysis

Plasmid pUC19 was positively supercoiled by adding chloroquine to nicked plasmid and ligating with T4 ligase (NEB) followed by purification with a PCR purification kit (Qiagen). Plasmids were reacted with ISWI at an equimolar ratio for 5' minutes in the presence of 25 mM ATP. This solution was used to prepare specimens for AFM as described above.

Images were analyzed by counting the number of intersections between segments in the supercoiled plasmids. In plectonemic sections, the length of the plectoneme was divided by the dimension of a single intersection to determine the number of crossovers.

Results and Discussion

ISWI binding to DNA

Chromatin remodelling enzymes have been shown to promote the generation of torsion that presumably catalyzes nucleosome repositioning¹⁷. This may also involve topological changes and some remodelers have been shown to induce supercoils in DNA substrates^{6,18} that could mobilize nucleosomes or provoke unwrapping¹⁹. Recent single molecule experiments have shown that in the SWI/SNF family the generation of torsion is coupled to a DNA loop formation^{6,7,18}. Although ISWI is a similar DNA translocase¹² which is hypothesized to push loops of DNA onto and around nucleosomes⁵, this has not yet been tested with single molecule experiments²⁰.

To study the form of the ISWI/DNA complex (i.e. to determine whether the DNA is wrapped on the enzyme or gathered into a supercoiled loop), an AFM was used to image the protein bound to linear 900 bp DNA in the absence of ATP. ISWI proteins do not require ATP for binding to DNA or chromatin^{12,14}. In the representative individual protein complexes bound to DNA shown in Fig. 1b, no DNA loop emanates from the complexes although the DNA is frequently sharply bent by the enzyme. The average angle between the DNA segments entering and exiting the complexes was about 120° (Fig. 1e). In fact the apparent contour length of the DNA was an average of 38 nm (113 bp) shorter in presence of ISWI (Fig. 1c), suggesting that the DNA was actually wrapped about the enzyme. Similar AFM images of another complete chromatin remodeling enzyme, the RSC complex, did not show any change in the length of the DNA molecule⁶.

If the DNA were wrapped around two thirds of the protein, then the length of DNA should be slightly greater than a two-thirds circumferential arc around the protein. Individual protein molecules were measured in AFM images both associated with DNA and isolated on the poly-L-ornithine-coated mica surface (Fig. 1). The radius of the protein increased from 7.8 to 11.2 nm upon association with DNA which could be due to the DNA encircling the protein (Fig. 2). Using a simple geometrical model of a filament wrapping two-thirds of a disc and assuming that the wrap has a radius of 9.6 nm (radius of ISWI alone, 7.8 nm, plus one-half of the difference with and without protein, 1.8 nm), one can estimate that approximately 40 nm of DNA would be required. This value is in rough agreement with the DNA contour length reduction observed in presence of ISWI. DNA wrapping would not seem conducive to DNA translocation, however wrapping might explain the ability of chromatin remodelling ATPases to alter the superhelicity of DNA¹². Indeed the CSB protein, which is a member of the SWI2/SNF2 family of ATP-dependent chromatin remodeling factors, has also been shown to wrap DNA without ATP. It was hypothesized that CSB functions as a dimer which has also been suggested for ISWI⁵. However, for AFM imaging the protein and DNA were mixed in equimolar amounts (see methods) and no

clustering of protein was observed that would suggest high cooperativity. In addition, the dimensions of particles measured in these AFM images average only 7.8 nm in diameter and 1 nm in height to give a cylindrical volume of 198 nm³. Considering that proteins generally have a partial specific volume of 0.74 cm³/g and that ISWI has a molecular weight of 140 kDa, the protein should occupy 260 nm³. Therefore the particles measured using AFM are most likely monomeric.

DNA translocation by ISWI

In presence of 25 μM ATP, the complex was often found at the end of a DNA molecule (71 end-bound out of 91 total), whereas without ATP the complex was more randomly positioned along the DNA (15 end-bound out of 109 total). This observation suggests that the enzyme moves along the DNA and does not dissociate rapidly from its ends. In this ensemble of molecules with ATP in which the enzyme was frequently positioned at one end of the linear DNA, loosely bound supercoiled loops were observed (Fig. 1c). When the complex was observed at a mid-position along the molecule, the DNA loop appeared highly supercoiled. This is in striking contrast to images taken in absence of ATP in which DNA was tightly associated with the protein complexes distributed randomly along the DNA. The average contour length of the DNA fragment with ISWI bound in the presence of ATP was 106 nm shorter than the naked DNA and matches length contractions detected in stretched DNA at similar ATP concentrations using magnetic tweezers (unpublished data). It is difficult to imagine that a single enzyme can spool 300 bp of DNA onto itself and seems more likely that it must create a loop in the DNA. This data supports the idea that ISWI can utilize ATP to produce loops in DNA by acting as a DNA translocase. DNA binding increases with template length up to about 40 bp and translocation on nucleosomal templates has been observed up to around 50 bp beyond the nucleosome boundary and required an intact 3'-5' strand¹². ATP-dependent creation of such loops is widely hypothesized to underlie nucleosome repositioning phenomena^{14,17} and has been shown using single molecule experiments for the RSC chromatin remodelling complex^{6,7}. These AFM data support the idea that ATP binding and hydrolysis may drive ISWI through a contractile cycle that translocates DNA¹⁴.

To corroborate the AFM experiments, the extension of single DNA molecules exposed to ISWI with and without ATP was monitored using tethered particle motion assays^{15,21} (Fig. 3a). In the absence of protein, a microsphere tethered to a glass slide through a single DNA molecule exhibits Brownian motion limited by the length of the tether. The average distance of the microsphere from the anchor point is an observable indication of the tether length that may change as DNA binding proteins modify topology²².

Figure 3b is a plot of the two-dimensionally projected distances of the microsphere (bead) from the anchor point versus time which in the absence of ISWI are broadly scattered. The 4 second time average of this signal is a stable trace (red solid line). Adding 20 nM ISWI caused abrupt transitions between two distinct lengths (Fig. 3c). It is noteworthy that in the presence of protein, the tether is slightly shorter than the tether without protein. This has also been seen in other tethered particle motion measurements which are sensitive enough to detect single bends²³⁻²⁵. This suggests that ISWI may have two binding modes, a loose association which bends the DNA and another in which an amount of DNA roughly equivalent to that encircling a histone octamer is wrapped or looped by the protein²⁶. The calibration of TPM excursions for such a short length of DNA with the relatively large bead is difficult and therefore the length of the DNA segment corresponding to the transition could not be determined. However the scatter of positions for both states are distinct from that of beads stuck on the surface (see supplementary information figure S3). In addition the duration of the long and short tether lengths were ATP dependent.

Figure 3d shows a representative trace with transitions observed using 250 μM ATP in which three long intervals of the short tether state were observed. The durations of both the long and short tethers were exponentially distributed (see supplementary information figure S2) and constants representing the characteristic lifetimes for both states were determined by curve fitting. These constants are plotted versus ATP concentration in figures 3e and f. The long tether state progressively decreased as the ATP concentration increased. However the characteristic lifetimes of the short tether fell along a Michaelis-Menten curve with $K_m = 15.1 \pm 2.9 \mu\text{M}$ and $\tau_{\text{on max}} = 87.7 \pm 3.8 \text{ s}$. Such a K_m is consistent with micromolar values determined for other ATPases²⁷. This behavior suggests that ISWI is functioning like an ATP-driven relay that loops or wraps DNA.

Plasmid relaxation

Whether by wrapping or looping, ISWI has previously been shown to alter the supercoiling of chromatin templates¹³. As seen in figure 1, ISWI in the presence of ATP produced supercoiled loops. To further investigate this activity, positively supercoiled plasmids were generated, incubated with ISWI, and imaged using atomic force microscopy. Figure 4a shows representative supercoiled plasmids with large and small loops emanating from several nodes. After incubation with ISWI, most plasmids had fewer loops and crossovers (intersections between DNA segments) as shown for the two plasmids in Figure 4b. Ensembles of plasmids were imaged and crossovers were counted. Histograms of this data show that incubation with ISWI greatly reduced the number of nodes.

Conclusion

ISWI has been proposed to translocate on DNA by pulling a loop of extranucleosomal DNA onto the surface of the nucleosome²⁰. The loop might then be pumped across the nucleosome by the translocase domain which makes contact near the dyad¹¹. Similarly to ISW2¹⁴, ISWI bound randomly to linear DNA without ATP. Substantial shortening of the DNA indicated that about ten helical turns of DNA were wrapped around the monomeric protein in a manner reminiscent of that observed for the SWI/SNF family protein CSB¹⁸. As has been shown for the SWI/SNF family archetype RSC⁶, when ATP was available ISWI shifted toward the ends of the fragment and produced supercoiled loops. This was likely the result of ATP-driven translocation on the DNA, since the duration of protein-induced transitions in tethered particle motion assays was ATP-dependent. Recombinant drosophila ISWI appears to generate supercoiled, looped DNA templates by initially wrapping DNA and then negatively supercoiling it during translocation. Whether or not this behaviour persists when ISWI is assembled with its usual subunit partners remains to be investigated. Generally speaking, loop formation coupled to DNA translocation appears to be a common mechanism among chromatin remodelling enzymes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Human Frontier Science Program (LF), Italian Funding of Basic Research (DD, LF), and the Wellcome Trust (T.O-H).

References

1. Brown E, Malakar S, Krebs JE. Biochem Cell Biol. 2007; 85:444–462. [PubMed: 17713580]

2. Varga-Weisz PD, Becker PB. *Current Opinion in Genetics & Development*. 2006; 16:151–156. [PubMed: 16503135]
3. Cairns BR. *Curr Opin Genet Dev*. 2005; 15:185–190. [PubMed: 15797201]
4. Corona DF, Tamkun JW. *Biochim Biophys Acta*. 2004; 1677:113–119. [PubMed: 15020052]
5. Racki LR, Narlikar GJ. *Curr Opin Genet Dev*. 2008
6. Lia G, Praly E, Ferreira H, Stockdale C, Tse-Dinh YC, Dunlap D, Croquette V, Bensimon D, Owen-Hughes T. *Mol Cell*. 2006; 21:417–425. [PubMed: 16455496]
7. Zhang YL, Smith CL, Saha A, Grill SW, Mihardja S, Smith SB, Cairns BR, Peterson CL, Bustamante C. *Molecular Cell*. 2006; 24:559–568. [PubMed: 17188033]
8. Asturias FJ, Chung WH, Kornberg RD, Lorch Y. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:13477–13480. [PubMed: 12368485]
9. Leschziner AE, Saha A, Wittmeyer J, Zhang YL, Bustamante C, Cairns BR, Nogales E. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:4913–4918. [PubMed: 17360331]
10. Schwanbeck R, Xiao H, Wu C. *Journal of Biological Chemistry*. 2004; 279:39933–39941. [PubMed: 15262970]
11. Dang WW, Bartholomew B. *Molecular and Cellular Biology*. 2007; 27:8306–8317. [PubMed: 17908792]
12. Whitehouse I, Stockdale C, Flaus A, Szczelkun MD, Owen-Hughes T. *Molecular and Cellular Biology*. 2003; 23:1935–1945. [PubMed: 12612068]
13. Havas K, Flaus A, Phelan M, Kingston R, Wade PA, Lilley DMJ, Owen-Hughes T. *Cell*. 2000; 103:1133–1142. [PubMed: 11163188]
14. Fitzgerald DJ, DeLuca C, Berger I, Gaillard H, Sigrist R, Schimmele K, Richmond TJ, Embo J. 2004; 23:3836–3843. [PubMed: 15359274]
15. Finzi L, Dunlap D. *Methods Enzymol*. 2003; 370:369–378. [PubMed: 14712660]
16. Corona DFV, Langst G, Clapier CR, Bonte EJ, Ferrari S, Tamkun JW, Becker PB. *Molecular Cell*. 1999; 3:239–245. [PubMed: 10078206]
17. Becker PB. *Embo J*. 2002; 21:4749–4753. [PubMed: 12234915]
18. Beerens N, Hoeijmakers JH, Kanaar R, Vermeulen W, Wyman C. *J Biol Chem*. 2005; 280:4722–4729. [PubMed: 15548521]
19. Li G, Levitus M, Bustamante C, Widom J. *Nat Struct Mol Biol*. 2005; 12:46–53. [PubMed: 15580276]
20. Cairns BR. *Nature Structural & Molecular Biology*. 2007; 14:989–996.
21. Hong Y, Landick R, Gelles J. *Biophysical Journal*|*Biophysical Journal*. 1994; 67:2468–2478.
22. Nelson PC, Zurla C, Brogioli D, Beausang JF, Finzi L, Dunlap D. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys*. 2006; 110:17260–17267. [PubMed: 16928025]
23. Pouget N, Turlan C, Destainville N, Salome L, Chandler M. *Nucleic Acids Res*. 2006; 34:4313–4323. [PubMed: 16923775]
24. Tolic-Norrelykke SF, Rasmussen MB, Pavone FS, Berg-Sorensen K, Oddershede LB. *Biophysical Journal*. 2006; 90:3694–3703. [PubMed: 16500964]
25. Zurla C, Samuely T, Bertoni G, Valle F, Dietler G, Finzi L, Dunlap DD. *Biophysical Chemistry*. 2007; 128:245–252. [PubMed: 17543441]
26. Luger K. *Curr Opin Genet Dev*. 2003; 13:127–135. [PubMed: 12672489]
27. Brewer BJ, Martin SR, Champoux JJ. *Journal of Biological Chemistry*. 1983; 258:4496–4502. [PubMed: 6131894]

Figure. 1.

AFM data on the ISWI/DNA complex. a) AFM image of a bare linear DNA molecule. b) AFM image of an ISWI/DNA complex in absence of ATP. c) AFM image of two ISWI/DNA complexes in presence of 25 μ M ATP, one located at the middle and another at the end of the molecule. d) Histograms of the length of DNA flanking ISWI in AFM images such as those in *b* and *c*. The mean changes in extension upon binding of ISWI were: ≈ 39 nm with no ATP and ≈ 106 nm with 25 μ M ATP. e) Histogram of the angle (diagrammed in inset of *b*) between segments of DNA entering and exiting the complex in the absence of ATP.

Figure 2. Dimensions of ISWI particles in AFM images. Histograms of the height (a) and radius (b).

Figure 3.

Loops detected with tethered particle motion: a) A schematic diagram shows how 440 nm microspheres were tethered to a glass surface. b) Tethered particle motion without protein exhibits a broad scatter (green) of tether lengths that does not change during the course of the experiment. The 4-second moving average of this projected distance between the microsphere and the anchor point for a representative DNA tether without protein equals approximately 100 nm. c) Tethered particle motion with 60 μM ATP. Intervals exhibiting the broad scatter of the naked DNA alternate with intervals of considerably shorter projected tether lengths. d) Tethered particle motion with 250 μM ATP. The durations of short and long tether lengths were measured as τ_{on} and τ_{off} . e) The average duration of the shorter tether length as a function of ATP concentration. This red curve is a fitted Michaelis-Menten function with $K_m = 15.1 \pm 2.9 \mu\text{M}$ and $\tau_{on \max} = 87.7 \pm 3.8 \text{ s}$. f). The average duration of the longer tether length as a function of ATP concentration.

Figure 4.

ISWI reduced the supercoiling of positively supercoiled plasmids. a) Positively supercoiled plasmids appear randomly coiled and with plectonemic- and open-looped segments. b) After exposure to ISWI, these positively plasmids have few plectonemes and fewer loops. c) A histogram of the number of intersections counted for molecules in the ensemble is shown for plasmids with and without ISWI.