7580–7591 *Nucleic Acids Research, 2016, Vol. 44, No. 16* *Published online 12 May 2016*
doi: 10.1093/nar/gkw406

The Chd1 chromatin remodeler can sense both entry and exit sides of the nucleosome

Ilana M. Nodelman[1], Kyle C. Horvath[2], Robert F. Levendosky[2], Jessica Winger[2], Ren Ren[1], Ashok Patel[1], Ming Li[3], Michelle D. Wang[4,5], Elijah Roberts[1] and Gregory D. Bowman[1,2,*]

[1]T.C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218, USA, [2]Department of Biology, Johns Hopkins University, Baltimore, MD 21218, USA, [3]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA, [4]Department of Physics, LASSP, Cornell University, Ithaca, NY 14853, USA and [5]Howard Hughes Medical Institute, Cornell University, Ithaca, NY 14853, USA

Received September 28, 2015; Revised April 28, 2016; Accepted April 29, 2016

ABSTRACT

Chromatin remodelers are essential for establishing and maintaining the placement of nucleosomes along genomic DNA. Yet how chromatin remodelers recognize and respond to distinct chromatin environments surrounding nucleosomes is poorly understood. Here, we use Lac repressor as a tool to probe how a DNA-bound factor influences action of the Chd1 remodeler. We show that Chd1 preferentially shifts nucleosomes away from Lac repressor, demonstrating that a DNA-bound factor defines a barrier for nucleosome positioning. Rather than an absolute block in sliding, the barrier effect was achieved by altered rates of nucleosome sliding that biased redistribution of nucleosomes away from the bound Lac repressor site. Remarkably, in addition to slower sliding toward the LacO site, the presence of Lac repressor also stimulated sliding in the opposite direction. These experiments therefore demonstrate that Chd1 responds to the presence of a bound protein on both entry and exit sides of the nucleosome. This sensitivity to both sides of the nucleosome allows for a faster and sharper response than would be possible by responding to only the entry side, and we speculate that dual entry/exit sensitivity is also important for regularly spaced nucleosome arrays generated by Chd1 and the related ISWI remodelers.

INTRODUCTION

As the fundamental packaging unit of eukaryotic genomes, nucleosomes are involved in many basic cellular processes such as DNA replication, recombination, DNA repair and transcription. By virtue of their intimate association with DNA, nucleosomes can compete with other proteins for DNA binding. This limited accessibility to DNA allows events such as origin firing and gene expression to be regulated by the positions of nucleosomes on DNA (1–7). Well-defined nucleosome positions are common features of all eukaryotes, yet there are gaps in our understanding of how positions are established and maintained.

Chromatin remodelers are multidomain enzymes that play essential roles in assembly, disassembly and repositioning nucleosomes along DNA (8,9). Although disruption of histone-DNA contacts is driven by a common helicase-like ATPase motor (10), different remodeler families respond in unique ways to various characteristics of the nucleosome (11). Chd1 and ISWI remodelers, for example, slide nucleosomes directionally depending on available extranucleosomal DNA (12–15), whereas SWI/SNF-type remodelers shift the histone octamer regardless of DNA availability outside the nucleosome (16,17). For Chd1 and ISWI remodelers, the sensitivity to extranucleosomal DNA is believed to be responsible for repositioning mononucleosomes to the center of short DNA fragments, which appears to underlie the ability to generate evenly spaced nucleosomal arrays (18–22).

At least two remodeler elements are required for reading out extranucleosomal DNA to allow for directional nucleosome sliding. For Chd1, one key element is the DNA-binding domain, the deletion of which prevents centering of mononucleosomes (15). The DNA-binding domains of yeast Isw1 and Isw2 have been cross-linked at the nucleosome edge (23,24), and for both remodeler families this domain is generally believed to convey the presence of extranucleosomal DNA to the rest of the remodeler. Another key element, first observed in the crystal structure of the Chd1 chromo-ATPase fragment and shown to be conserved in both Chd1 and ISWI families (25), was also found to be required for mononucleosome centering by ISWI (26). This element has been termed 'NegC' in ISWI for a negative regulatory role in sliding (27) and the C-terminal 'bridge' in

*To whom correspondence should be addressed. Tel: +1 410 516 7850; Fax: +1 410 516 4118; Email: gdbowman@jhu.edu
Present address: Ashok Patel, Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India.

Chd1 for its structural organization across both lobes of the ATPase motor (25).

Although nucleosomes are the basic building blocks of chromatin, the chromatin environment also includes other factors that dynamically bind and release from DNA. Many transcription factor binding sites are found within and flanking nucleosomal footprints (28), and thus have the potential to influence remodelers that are sensitive to DNA both within and flanking the nucleosome. Indeed, nucleosome sliding by ISWI remodelers has been shown to be strongly influenced by bound transcription factors. Using nuclear extracts and purified ISWI remodelers, early studies showed that ATP-dependent remodeling promoted association of the transcription factors GAGA, TTF1 and Lac repressor to their binding sites (29–32). Using the ISWI remodeler NURF and the DNA-binding domain of Gal4, this cooperation with transcription factors was shown to stem from a barrier effect that biased nucleosome sliding away from bound proteins (33). The basis for such barrier effects, as well as the means by which chromatin remodelers sense and respond to transcription factors at the edge of the nucleosome have not been explored.

Given the biochemical and architectural similarities between Chd1 and ISWI remodelers, we hypothesized that Chd1 would also be sensitive to transcription factors bound adjacent to nucleosomes. Here we describe experiments that use the Lac repressor (LacI) system to test this prediction and investigate the nature of a barrier to nucleosome sliding.

MATERIALS AND METHODS

Protein constructs and purification

His-tagged *Saccharomyces cerevisiae* Chd1 variants were expressed and purified as previously described (25,34,35). Chd1 constructs included: an N- and C-terminally truncated variant containing the conserved chromo/helicase/DNA-binding domains (residues 118–1274), referred to throughout the text as Chd1; $Chd1_{RAM}$ (residues 118–1274), with the 121 residue RAM sequence (36) inserted between residues 1000 and 1001 (35); Chd1[ΔDBD] (residues 118–1014), which lacks the DNA-binding domain; and Chd1[GGS/bridge] (residues 118–1274), which possesses a $(gly\text{-}gly\text{-}ser)_{11}$ segment that replaces residues 884–918. The bridge substitution was made in a cysteine-variant background where all five natural cysteines were removed and A1117C was introduced. All histones were purified and reconstituted into histone octamers as previously described (37). *Saccharomyces cerevisiae* histones were used for experiments shown in Figure 4A; *Xenopus laevis* histones were used for all other experiments. Lac repressor (residues 1–133) was expressed from the pET101D-LacI construct, which lacks the C-terminal tetramerization domain and forms a constitutive dimer (38). LacI was expressed in BL21 Star(DE3) cells that were induced with IPTG and harvested after overnight growth at 37°C. After purification by nickel affinity and size exclusion chromatography, LacI was stored at −80°C.

DNA template and nucleosome preparation

A symmetric and high affinity variation of LacO (5′-AATTGTGAGCGCTCACAATT) (39) was introduced into the 601 nucleosome positioning sequence (40) using PCR mutagenesis (see Supplementary Table S1 for primer sequences). LacO-containing 601 sequences were used as templates for PCR (see Supplementary Table S2 for primer sequences). DNA fragments were purified and incorporated into nucleosomes as previously described (35,37).

Nucleosome sliding and histone mapping

Mapping was carried out using *X. laevis* histone octamers with the H3(C110A) substitution to remove the naturally occurring cysteine, along with an H2B(S53C) substitution. After labeling nucleosomes with APB (41), sliding reactions were carried out in 1× slide buffer (20 mM HEPES–KOH, pH 7.6; 50 mM KCl; 5 mM $MgCl_2$; 0.1 mg/ml BSA; 10 mM DTT; 5% sucrose) using 150 nM nucleosome, 50 nM remodeler and 2 mM ATP. In reactions with nucleosomes containing LacO(+1) and LacO(−11) sites, LacI was added at a 5-fold molar excess over nucleosomes (750 nM), whereas for LacO(−6)-containing nucleosomes, LacI was added at a 32-fold molar excess (4.8 μM). After UV irradiation and processing (41), samples were separated on 8% polyacrylamide (19:1), 8M urea sequencing gels run at 65 W and visualized on a GE Typhoon 9410 variable mode imager. Rates of nucleosome sliding were calculated by fitting the fraction of shifted versus unshifted nucleosomes to the single-exponential function $y = a*(e^{-k*x}) + c$, where the fit parameters were a (normalized amplitude), c (constant), and k (rate constant, min^{-1}).

Nucleosome binding

Histone H4(A15C) was labeled with Cy3 maleimide and reconstituted into nucleosomes (26). Changes in Cy3 fluorescence intensity were monitored in a Horiba Jobin Yvon Fluorolog fluorometer with the sample chamber set at 25°C, excitation monochromator at 510 nm (5 nm slit width), and emission monochromator at 565 nm (5 nm slit width). Increasing amounts of Chd1 were titrated into solutions containing 5 nM nucleosome in 20 mM HEPES, pH 7.5; 5 mM $MgCl_2$; 0.1 mM EDTA; 5% (w/v) sucrose; 1 mM DTT; 0.02% Nonidet P40 Substitute (Roche); 0.1 mg/ml BSA; 1 mM adenosine 5′-(β,γ-imido)triphosphate (AMP-PNP) and 100 mM KCl. Titrations with LacI contained 500 nM LacI. The binding data was fit in KaleidaGraph (Synergy) to the following binding isotherm using nonlinear least squares regression,

$$Y = (A_1/(2*N))* \left(X + N + K_1 - \left((X + N + K_1)^2 - 4*X*N\right)^{1/2}\right) + A_2*X/(K_2 + X) + C$$

where Y is the signal intensity, X is the concentration of Chd1, A_X are the signal amplitudes, K_X are the dissociation constants, N is the nucleosome concentration and C is the signal from nucleosome alone. Titrations for each experimental condition were performed three or more times, with the average $1/K_{1/2}$ values and standard deviations reported in Figure 3.

Native gel sliding

Native gel sliding experiments that monitored Chd1[ΔDBD] activity were performed using nucleosomes reconstituted with *S. cerevisiae* histones and conditions as previously described in (15). LacI was added at a final concentration of 150 nM (6-fold excess over nucleosomes), and the stop solution additionally contained 1.5 mM IPTG. All other native gel sliding experiments utilized *X. laevis* nucleosomes and were carried out as previously described (35). Experiments with Chd1[GGS/bridge] used nucleosomes reconstituted with either [FAM]40N40 or [Cy3]0N80[LacO-11R,FAM] DNA, with the addition of 750 nM LacI (5-fold excess over nucleosomes). For the competition experiments, 50 nM Chd1 was combined with one of the following: 150 nM [Cy5]0N80 alone; 150 nM [Cy5]0N80 + 150 nM [Cy3]0N80[LacO-11R] ± LacI (750 nM); or 300 nM [Cy3]0N80[LacO-11R] + LacI (1.5 μM). These samples were mixed in a 20 μl volume, with 1 μl removed at each time point to a quench solution containing 5 mM EDTA and plasmid DNA possessing a single LacO site. Nucleosome samples were separated by native acrylamide gels, visualized using a GE Typhoon 9410 variable mode imager, and the relative intensities of different nucleosome species were obtained using ImageJ (http://imagej.nih.gov/ij/). Fits to the data shown in Figure 5 were either single (for Cy3 0N80[LacO-11] nucleosomes in the presence of LacI) or double exponential fits (for all other conditions). For the double exponential fits, the slower rates were similar (6- to 8-fold slower than the faster rate), and only the faster rate is reported.

Restriction digestion

An EcoRI restriction cut site was introduced within the 601 positioning sequence by PCR amplification, 10–15 bp from the left edge of the 601 sequence. Digestions of 150 nM fluorescently labeled [FAM]0N70[LacO-11R,Cy5] nucleosomes were carried out using 2 U/μl of EcoRI-HF (NEB) in 1×CutSmart buffer at 30°C. After pre-incubation of Chd1 (100 nM), nucleosomes (150 nM) and ±LacI (1.2 μM) for 5 min, EcoR1-HF was added for 2 min to digest free DNA, followed by ATP addition to a final concentration of 2.5 mM. Control reactions showed that free DNA was digested within ∼0.5 min. Time points were taken by transferring 1 μl of each 25 μl reaction to 9 μl of quench (SDS-PAGE loading buffer with 40 mM EDTA). Samples (3 μl) were separated by SDS-PAGE (18% acrylamide), visualized using a GE Typhoon 9410 variable mode imager, and quantified using ImageJ (http://imagej.nih.gov/ij/). As previously described for this assay (42), not all DNA was digested after extended incubation, and all samples were normalized to the same range of nucleosomal DNA that could be cleaved by EcoRI based on samples remodelled with Chd1 in the absence of LacI. Rates were calculated with single exponential fits to data as described above.

Kinetic modelling

To model the process of nucleosome repositioning, a kinetic model was formulated with forward and backward transitions between five sequential states representing the observed high-occupancy nucleosome positions determined by histone mapping, as shown in Figure 6F. Numerical integration of the resulting series of ordinary differential equations was used to calculate the time-dependent occupancy of each state, starting from the initial condition of 100% 'C' and 0% occupancy for the other four species. Kinetic parameters were estimated from the experimental data by fitting the fraction of each nucleosome species at each time point to the output of the kinetic simulations through minimization (BFGS method) of the sum of the square differences. Sensitivity to initial parameter values during the fit were tested by randomly sampling initial rates between 0.1 and 5.0 min^{-1}. We also tested for robustness by randomly sampling the value of each of the experimental data points from a Gaussian distribution determined by the point's experimental error estimate, as well as randomly varying the time value for each point, also with a Gaussian distribution using a 0.5 s standard deviation. In all, >40 000 fits for each experimental condition were analysed to determine the space of transition rate constants that were compatible with the experimental data (see Supplementary Figures S1 and S2).

RESULTS

LacI limits the extent and rate of nucleosome sliding by Chd1

To test whether nucleosome sliding by Chd1 is sensitive to a protein bound at the entry site of the nucleosome, we generated mononucleosomes with the LacO binding site on one side. We chose Lac repressor (LacI) because we desired a foreign transcription factor that would bind and occlude a defined segment of DNA without making specific interactions with Chd1, which should allow us to determine which portions of DNA may be important for Chd1 activity.

Remodeling reactions were carried out with a previously characterized and truncated form of *S. cerevisiae* Chd1 (residues 118–1274, hereafter referred to as Chd1) possessing the conserved chromodomains, ATPase motor, and DNA-binding domain (15,34). Since Chd1 preferentially slides end-positioned nucleosomes toward available extranucleosomal DNA, we generated 601-based mononucleosomes (40) having zero bp of extranucleosomal DNA on one side and 80 bp on the other (referred to as 0N80). To see if the presence of LacI would influence nucleosome repositioning by Chd1, we first tested a location where the entire 20 bp LacO site was on the flanking 80 bp DNA and adjacent to the edge of the 601 sequence (denoted 0N80[LacO + 1R]).

To precisely identify the positions of the remodeled products, we analyzed reactions by histone mapping. With this technique, the photoactivatable cross-linker azidophenacyl bromide (APB), attached via a cysteine substitution at position 53 of histone H2B (H2B-S53C), reacts with one strand of DNA approximately 18 bp from the nucleosome edge, and can be used to monitor the location of the histone octamer before and after nucleosome sliding (41). In the absence of LacI, histone mapping showed that Chd1 shifted these nucleosomes up to 54 bp onto the extranucleosomal DNA (Figure 1A). In contrast, in the presence of LacI, the extent of nucleosome repositioning by Chd1 was significantly limited, with the majority of nucleosomes shifting

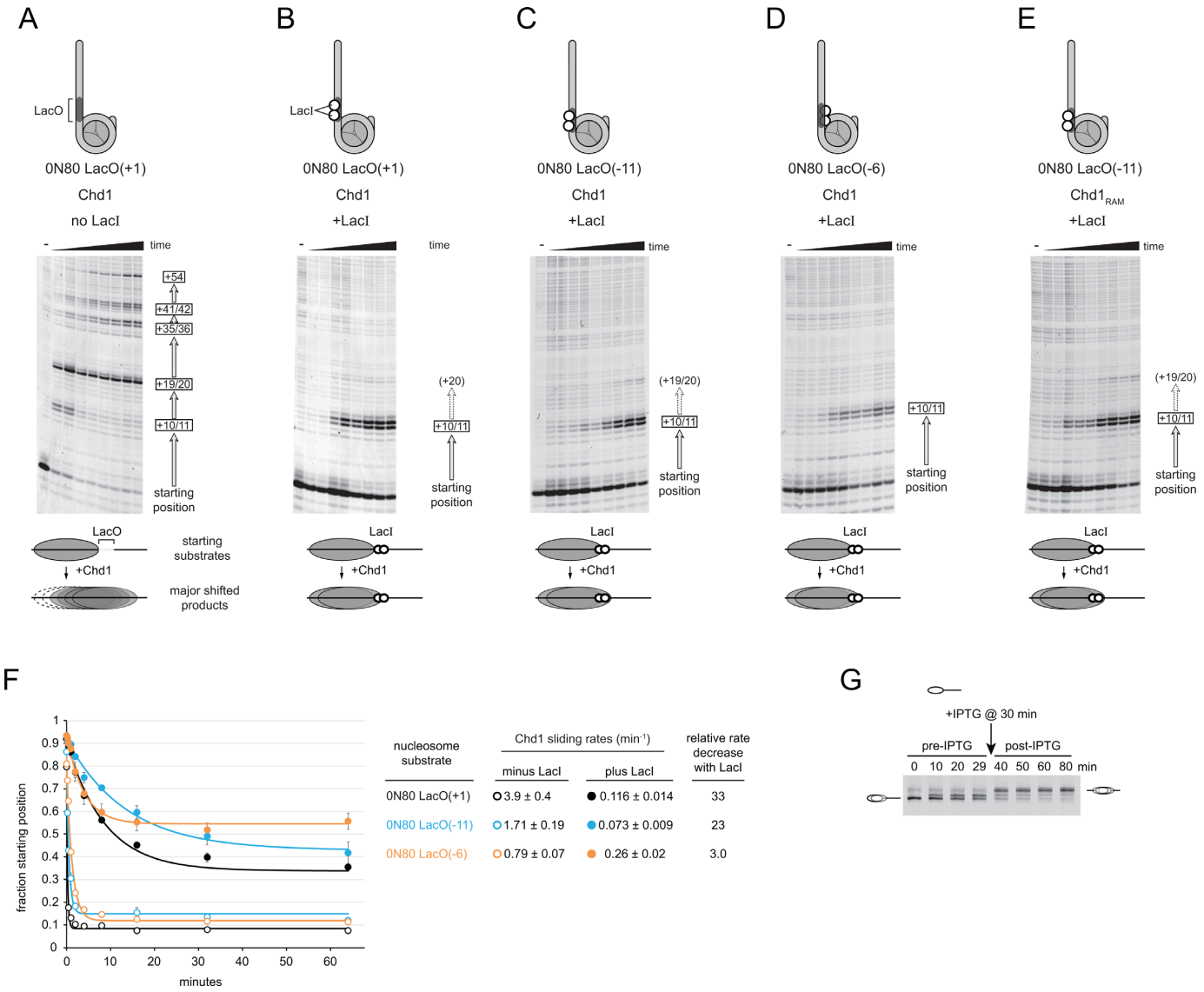

Figure 1. The presence of Lac repressor limits the extent and rate of nucleosome sliding by Chd1. Repositioning 0N80 nucleosomes by Chd1, monitored by histone mapping at H2B(S53C). Shown are representative examples of sliding reactions carried out in the absence of LacI using 0N80[LacO+1] nucleosomes (**A**), and reactions carried out in the presence of LacI for 0N80 nucleosomes with LacO binding sites located +1 bp (**B**), −11 bp (**C**) or −6 bp (**D**) from the right edge of the nucleosome. Top cartoons illustrate the relative positions of LacO and LacI on the nucleosome, whereas bottom cartoons summarize the major nucleosome positions resulting from each experiment. (**E**) Extended reach of the Chd1 DNA-binding domain does not alter the inhibitory effects of LacI. Nucleosome sliding was carried out using a variant of Chd1 containing a 121-residue insertion between the ATPase motor and DNA-binding domain, called $Chd1_{RAM}$. Shown is a representative of three experiments. Time points for reactions in (A–E) were 0, 0.25 min (for LacO(−6) and LacO(−11) only), 0.5, 1, 2, 4, 8, 16, 32, 64 min. (**F**) Quantification of the nucleosome sliding experiments shown in (A–D), revealing a more rapid disappearance of the starting material when remodeled in the absence (open circles) compared with the presence (filled circles) of LacI. Each point represents the average values from two or more experiments, with error bars showing the range of values from two measurements, or the standard deviations for three or more measurements. Rate constants were calculated from single exponential fits to the data, with the error given as the standard deviation from averaging calculated rate constants. (**G**) Sliding in the presence of LacI does not permanently disrupt nucleosomes. Remodeling of 0N80[LacO-11R] nucleosomes was monitored by native PAGE. After 30 min of sliding, IPTG was added to release LacI, and the change in nucleosome mobility shows that this addition was sufficient for removing the LacI barrier.

only 10 to 11 bp from the starting position (Figure 1B). The 10–11 bp shift effectively pulled about half of the LacO site onto the nucleosome. To see whether LacI on the repositioned LacO site was responsible for blocking further sliding, we generated end-positioned nucleosomes with a preshifted binding site (0N80[LacO-11R]) as well as a site with a different phasing of LacO (0N80[LacO-6R]). In the presence of LacI, these nucleosomes showed similar patterns of repositioning by Chd1, with about half of the nucleosomes shifting by only 10–11 bp (Figure 1C and D). These results show that LacI can act as a barrier to nucleosome repositioning by Chd1, yet the similar patterns of nucleosome sliding with the three LacO positions suggest that Chd1 was not blocked by a specific location of LacI.

In addition to limiting the range of repositioning, the presence of LacI also markedly decreased the rate of nu-

cleosome sliding by Chd1 (Figure 1F). The most dramatic rate differences were for 0N80[LacO+1R] and 0N80[LacO-11R] nucleosomes, which were shifted 33-fold and 23-fold more slowly in the presence of LacI, respectively. LacI also significantly decreased the sliding rate for 0N80[LacO-6R] nucleosomes, though the difference was a more modest 3-fold slower sliding with LacI.

Given the limited repositioning in the presence of LacI, we considered the possibility that action of Chd1 on LacI-bound nucleosomes might create inactive, non-nucleosomal products. To examine this, we monitored sliding reactions before and after addition of IPTG to release LacI from nucleosomes. Consistent with histone mapping experiments, native PAGE showed that LacI restricted nucleosome repositioning by Chd1 (Figure 1G). After 30 min of ATP-dependent nucleosome sliding, IPTG was added to the reaction, which allowed Chd1 to shift nucleosomes to a more central location on the DNA fragment. This result indicates that nucleosome sliding in the presence of LacI does not permanently disrupt histone-histone or histone-DNA interactions.

Extending the reach of the Chd1 DNA-binding domain does not diminish sensitivity to LacI

Binding of LacI at the nucleosome edge limited the extent and rate of nucleosome sliding by Chd1. Since the Chd1 DNA-binding domain is expected to interact with extranucleosomal DNA, we wondered whether LacI somehow constrained the placement of the DNA-binding domain relative to the rest of the remodeler. We previously inserted a 121 residue, intrinsically disordered peptide segment, called RAM, between the DNA-binding and ATPase domains of Chd1. This RAM insertion still allowed Chd1 to reposition nucleosomes, and in the context of the foreign, sequence-specific DNA-binding domain of AraC, gave the Chd1-AraC fusion remodeler a longer reach to specific binding sites on extranucleosomal DNA (35). Here, $Chd1_{RAM}$ (with a native DNA-binding domain) was incubated with the same 0N80[LacO-11R] nucleosomes under sliding conditions in the presence and absence of LacI. Histone mapping of these samples revealed that, similar to Chd1, the majority of nucleosomes were shifted just 10–11 bp (Figure 1E). Although we could not confirm positioning of the DNA-binding domain on extranucleosomal DNA, the similar LacI effects for $Chd1_{RAM}$ shows that a more flexible attachment of the DNA-binding domain is insufficient for bypassing the inhibitory effect of a protein bound on entry/exit DNA.

Availability of exit DNA is critical for the barrier effect of LacI

Although LacI clearly interfered with nucleosome sliding, Chd1 shifted nucleosomes 10 to 11 bp regardless of the initial LacO placement. We reasoned that the observed barrier activity of LacI may stem from the newly generated extranucleosomal DNA on the exit side. As Chd1 shifts the LacO site toward the nucleosome by 10–11 bp, the emergence of ∼1 turn of exit DNA may be sufficient for the remodeler to engage the opposite face of the nucleosome, which could stimulate nucleosome sliding in the opposite direction. In this sense, the newly generated exit DNA would then serve as entry DNA, with the LacO site, on the opposite side of the nucleosome, poised to be shifted off the histone octamer and back onto the flanking DNA.

For this scenario, shifting nucleosomes alternately toward and away from the LacO site would repopulate the initial, end-positioned location of the histone octamer on DNA, and would be consistent with the significant fraction (∼50%) of 0N80 nucleosomes that appeared to remain at the starting position throughout the reaction (Figure 1). To determine whether the entire nucleosome population was continually sliding back-and-forth, an EcoRI cut site was introduced close to the zero side of 0N80[LacO-11R] nucleosomes. This site was designed to be initially occluded by the histone octamer, becoming exposed upon movement of the nucleosome toward the LacO sequence. Reactions carried out in the absence of ATP showed a slow rate of digestion, consistent with protection of the EcoRI site by the nucleosome (Figure 2A). In the absence of LacI, addition of Chd1 and ATP promoted rapid digestion, which agrees with the fast nucleosome repositioning away from the shorter DNA end (Figures 1A, F and 2A). As expected, addition of LacI slowed down DNA cutting. Importantly, despite the slower rate of digestion, the presence of LacI did not diminish the population of DNA accessible to EcoRI, supporting the hypothesis that Chd1 continually shifts nucleosomes back-and-forth in the presence of LacI.

To test the idea that the barrier action requires available DNA on the other side of the nucleosome, we generated (−10)N80[LacO-11R] nucleosomes. For these nucleosomes, the short DNA end was at a more internal position on the (−10) side, and therefore would require a 20 bp shift to produce just 10 bp of flanking DNA. If the limited 10–11 bp shift of 0N80[LacO-11R] resulted from the generation of ∼1 turn of DNA on the exit side, we reasoned that the (−10)N80[LacO-11R] nucleosomes would have to move ∼20 bp to expose a similar length of DNA on the exit side. When LacI was not present, Chd1 shifted the (−10)N80[LacO-11R] nucleosomes away from the recessed (−10) bp side and onto the 80 bp side (Figure 2B). Similar to 0N80[LacO] nucleosomes, sliding of the (−10)N80[LacO-11R] nucleosomes was restricted in the presence of LacI, yet the distribution was clearly shifted to favor both 10–11 bp and 19–20 bp products. Although the majority of (−10)N80 nucleosomes shifted farther than 0N80 nucleosomes, the final products were quite similar, with approximately equal amounts of nucleosomes having ∼0 and ∼10 bp of extranucleosomal DNA on the side opposite the LacO site (Figure 2C). These results are consistent with LacI slowing the rate that Chd1 shifted nucleosomes toward LacI, relative to the rate of sliding nucleosomes in the opposite direction. This means that LacI does not prevent sliding by Chd1, but indicates that when exit side DNA is available, sliding in the opposite direction is more favorable.

LacI does not appear to antagonize initial engagement of Chd1 at SHL2 on the nucleosome

Previously, DNA-gap and cross-linking studies showed that nucleosome sliding by Chd1, ISWI and SWI/SNF-type re-

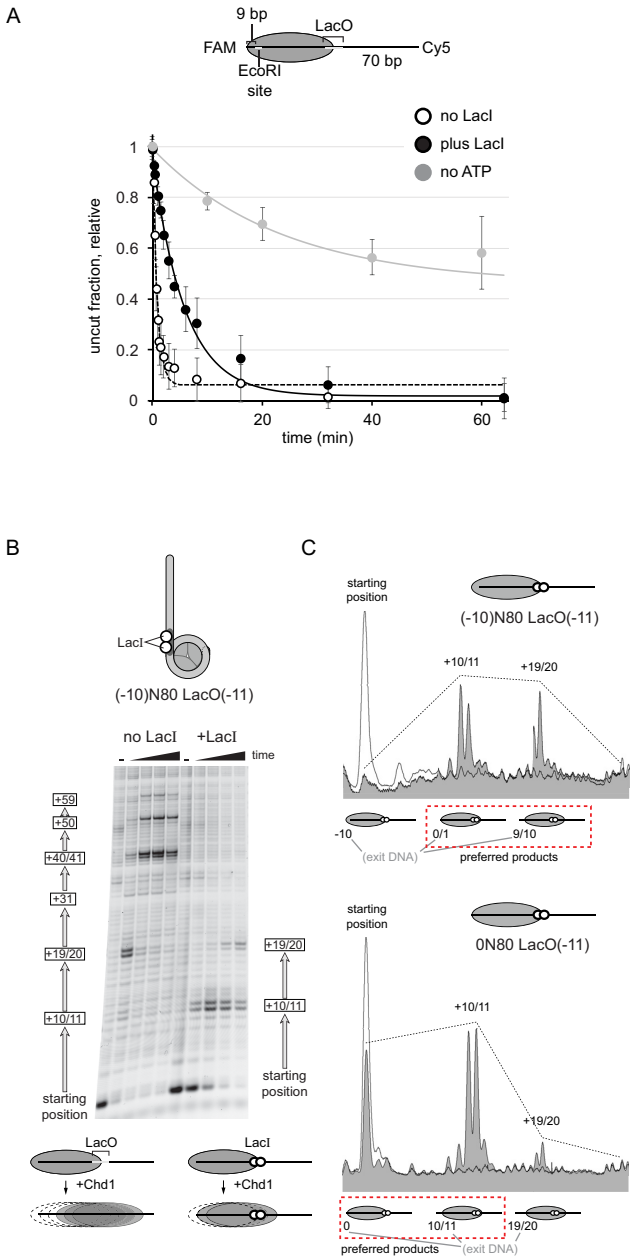

Figure 2. Exit side DNA defines the extent that Chd1 slides nucleosomes in the presence of Lac repressor. (**A**) Nucleosomes are dynamically shifted back-and-forth by Chd1 in the presence of LacI. End-positioned 0N70 nucleosomes containing a LacO(−11) site on the 70 bp side and an EcoRI cut site just inside the 0 bp side were digested by EcoRI and monitored by SDS-PAGE. The slower rate of digestion in the absence of ATP (gray) demonstrates that the EcoRI site is initially buried. In the presence of Chd1 and ATP, the same fraction of nucleosomal DNA becomes cleaved in the presence (filled circle) or absence (open circle) of LacI, demonstrating dynamic repositioning by Chd1 in the presence of LacI. Based on single exponential fits to the data, the sliding rate in the presence of LacI was calculated to be 7-fold slower than in the absence of LacI. Error bars indicate the standard deviations from five or more independent experiments. (**B**) Comparison of nucleosome sliding reactions carried out in the absence and presence of LacI, using (−10)N80[LacO-11R] substrates. Time points for these experiments were 0, 1, 4, 16, 64 min. (**C**) Comparison of the preferred distributions of nucleosome positions for 0N80 and (−10)N80 nucleosomes when Chd1 sliding was carried out in the presence of LacI. White peaks show zero time points and gray peaks are the nucleosome positions at 64 min time points.

modelers is driven by action of the ATPase motor at superhelical location 2 (SHL2), an internal position on the nucleosome located ∼20 bp from the dyad (15,24,43–45). Although the LacO sites at the nucleosome edge are ∼50 bp away from SHL2, we considered the possibility that the presence of LacI may impair nucleosome recognition, which could indirectly weaken association of the ATPase motor at SHL2. To determine the extent that binding at SHL2 might be disrupted by the presence of LacI at the edge of the nucleosome, we used a fluorescence-based assay that monitors remodeler binding near the histone H4 tail, which is adjacent to SHL2 (26). Binding reactions were carried out in the presence of the non-hydrolyzable ATP analog, adenylyl imidodiphosphate (AMP-PNP).

The affinity of Chd1 for 0N80[LacO-11R] nucleosomes was not significantly diminished by LacI (Figure 3). This insensitivity to LacI may stem in part from the relatively long flanking DNA of the 0N80 nucleosome, which could allow the DNA-binding domain to assist the ATPase motor in nucleosome binding. To eliminate interactions with extranucleosomal DNA beyond the LacI binding site, we generated 0N11[LacO-11R] nucleosomes, which had a short DNA segment that extended just two base pairs farther than the LacO sequence. Interestingly, with this shortened extranucleosomal DNA, the addition of LacI reduced Chd1 binding to 0N11[LacO-11R] nucleosomes by ∼4-fold, which suggests that the presence of LacI can compete with Chd1 under conditions where flanking DNA is limited. To evaluate how much Chd1 may rely upon extranucleosomal DNA for nucleosome binding, we also measured Chd1 affinity for a 1N1 nucleosome, which lacked a LacO site. Affinity for the 1N1 nucleosome was ∼10-fold weaker than for the 0N11 nucleosomes in the absence of LacI (Figure 3). With limited flanking DNA, LacI therefore appeared to disrupt most of the favorable interactions with extranucleosomal DNA, with the affinity of Chd1 for 0N11[LacO-11R] plus LacI just ∼2-fold above the affinity for 1N1 nucleosomes.

Unexpectedly, LacI increased the affinity of Chd1 for LacO(−6) nucleosomes (Figure 3). Although modest (∼2-fold), this increase was observed for nucleosomes with both excess and limiting DNA extending beyond the LacO site. While these results highlight a connection between SHL2 binding and DNA availability near the entry/exit site of the nucleosome, the opposite effects of LacI bound to LacO(−11) and LacO(−6) sites suggest that the reduced sliding rates with LacI bound did not simply result from blocking the initial engagement of the Chd1 ATPase motor with the nucleosome.

LacI interferes with Chd1 action independently of sensing extranucleosomal DNA

Given its role in sensing DNA availability, we expected that the Chd1 DNA-binding domain would be required for a barrier response. To test this idea, we performed sliding reactions using a Chd1 construct lacking the DNA-binding domain (called Chd1[ΔDBD]), which shifts nucleosomes without regard to extranucleosomal DNA (15,34). In the absence of LacI, Chd1[ΔDBD] produced a redistribution of nucleosome bands by native PAGE (Figure 4A). If LacI were sensed via the DNA-binding domain alone, then no

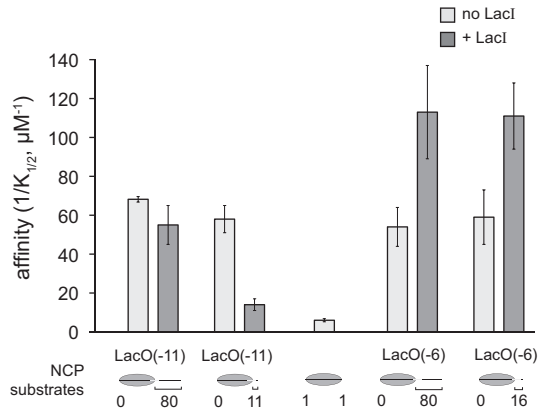

Figure 3. Chd1 can bind to nucleosomes at SHL2 in the presence of Lac repressor. Interactions between Chd1 and Cy3-labeled H4(A15C) nucleosomes were measured in the presence of the ATP analog AMP-PNP by monitoring changes in Cy3 fluorescence with increasing Chd1 concentrations. Error bars indicate the standard deviations from three or more fits to independent experiments.

differences in nucleosome positions would be expected for this reaction. However, addition of LacI clearly changed the nucleosome sliding pattern, consistent with a more limited positioning. Thus, in contrast to our expectations, Chd1 was clearly sensitive to the presence of LacI independently of the Chd1 DNA-binding domain.

For the ISWI remodeler, it was recently shown that directional sliding could be perturbed by disruption of a segment immediately C-terminal to the ATPase motor, called NegC in ISWI and the bridge in Chd1 (26). We therefore sought to test whether LacI can still affect the distribution of nucleosomes for a similarly disrupted Chd1 variant. Substitution of this element with a flexible $(gly\text{-}gly\text{-}ser)_{11}$ segment appeared to make ISWI insensitive to the presence or absence of extranucleosomal DNA and thus lose directionality (26), and we therefore generated a construct with a similar gly-gly-ser substitution (called Chd1[GGS/bridge]). As for ISWI, the GGS/bridge substitution in Chd1 disrupted the normal positioning of centered and end-positioned nucleosomes (Figure 4B). However, despite the defective responses to available flanking DNA, LacI still limited the observed distribution of nucleosome positions, for both Chd1 and Chd1[GGS/bridge] (Figure 4C). Thus, even when Chd1 lacks either of two basic elements required for responding to extranucleosomal DNA – the DNA-binding domain or the bridge – LacI still influences the pattern of nucleosome repositioning.

LacI destabilizes Chd1-nucleosome complexes during remodelling

Since LacI does not appear to directly disrupt initial engagement of Chd1 with the nucleosome, the presence of LacI may interfere with intermediate steps in the nucleosome sliding cycle. Slower rates of sliding could arise from new rate-limiting steps during DNA translocation, where Chd1 might be sequestered on LacI-bound nucleosomes. Alternatively, slower sliding could arise from more rapid dissociation of Chd1 from nucleosomes prior to productive sliding. To distinguish between these possibilities, we performed competition experiments, where Chd1 was given a 50/50 mixture of nucleosomes with and without the LacO sequence (labeled with Cy3 and Cy5, respectively), and the rate of nucleosome sliding was monitored by native PAGE. In the absence of LacI, progress curves for these two populations of nucleosomes were indistinguishable (Figure 5, open circles), giving rates of 0.5 ± 0.2 and 0.6 ± 0.2 min^{-1}. When LacI was included, the LacO-containing Cy3 nucleosomes were shifted more slowly, as expected (0.045 ± 0.011 min^{-1}, filled pink circles). Interestingly, the non-LacO Cy5 nucleosomes showed a distinct profile that suggested faster sliding (0.8 ± 0.2 min^{-1}, filled blue circles). Faster sliding of non-LacO nucleosomes would result from a more rapid dissociation from LacO-containing nucleosomes. To estimate the sensitivity of this competition experiment, similar reactions were performed using non-LacO Cy5 nucleosomes only, equivalent to half the nucleosome concentration of the Cy3/Cy5 mixture. Theoretically, with half of the nucleosome substrate concentration, the observed sliding rate should increase if the K_M is higher than the nucleosome concentration, approaching a maximum 2-fold rate increase. Under these conditions, the isolated non-LacO nucleosomes yielded a strikingly similar profile to that observed in the competition experiment when LacI was present, with an equivalent rate of 0.8 ± 0.2 min^{-1} ('X' symbol). Given the relatively high error, the calculated rates for non-LacO Cy5 nucleosomes alone were not statistically distinct from rates of LacO/non-LacO competition experiments. Despite this limitation, however, it is clear that sliding of non-LacO nucleosomes was not inhibited and instead may be increased by the presence of LacO-nucleosomes bound to LacI. Therefore, Chd1 does not become sequestered on nucleosomes containing LacI, and is likely released more rapidly after initial engagement with the nucleosome.

LacI increases the rate that Chd1 slides nucleosomes away from LacO

Experiments presented in Figures 1 and 2 used end-positioned nucleosomes, where the LacO site was on the only available DNA flanking the nucleosome. Although Chd1 initially shifted these end-positioned nucleosomes toward the side where LacI was bound, the requirement for ~10–11 bp of DNA available on the other side of the nucleosome suggested that if given the choice, Chd1 would likely shift nucleosomes away from bound sites. To investigate this, we tested sliding behavior of 40N40 nucleosomes. Nucleosomes were prepared with a LacO site on the left or the right side, and sliding reactions were performed in the presence or absence of LacI. Histone mapping revealed that in contrast to the relatively symmetric distribution of nucleosomes in the absence of LacI, Chd1 markedly shifted nucleosomes away from LacI-bound LacO sites (Figure 6A,B).

Interestingly, the redistribution of nucleosomes appeared to occur more rapidly in the presence of LacI. To determine whether this effect was due to slower sliding toward LacO, we fit the data to a simple kinetic model defined by five major populations of nucleosomes: a central starting state ('C') flanked by two states on each side ('A', 'B' and 'D',

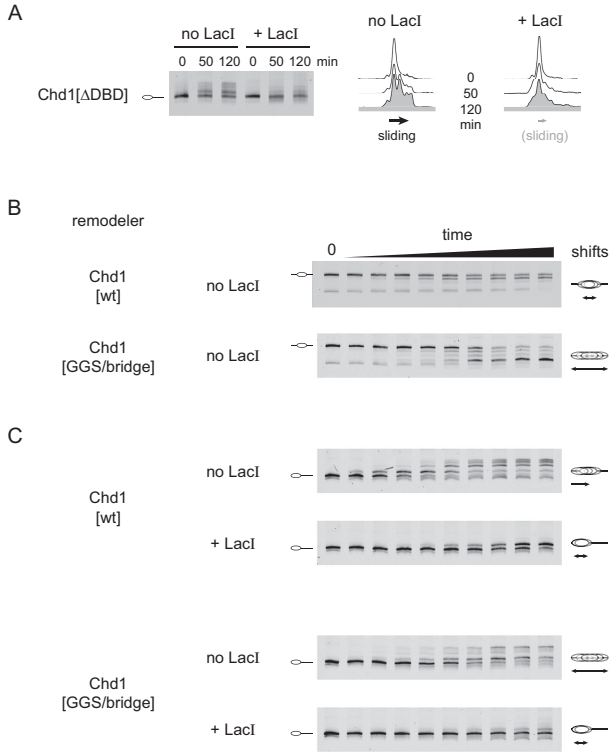

Figure 4. The ability of Chd1 to sense extranucleosomal DNA is not required to respond to a LacI barrier. (**A**) LacI diminished Chd1 sliding even in the absence of the Chd1 DNA-binding domain. Sliding experiments were performed with 0N80[LacO-11] nucleosomes using a Chd1 construct that lacked the DNA-binding domain (Chd1[ΔDBD]), carried out in the presence or absence of LacI. The left side shows a representative experiment, and the right side shows intensity profiles for each time point. (**B**) The bridge element of Chd1 is required for properly responding to extranucleosomal DNA. Nucleosome sliding experiments used precentered 40N40 nucleosomes. (**C**) LacI limits nucleosome sliding by the Chd1[GGS/bridge] variant. Shown are nucleosome sliding experiments using end-positioned 0N80[LacO-11] nucleosomes, carried out in the presence or absence of LacI. Time points for gels in (B) and (C) were 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 min. All experiments were analyzed by native acrylamide gels and carried out three or more times.

'E'), with each state linked to its neighbor through a forward and reverse rate (Figure 6C and D). Injection of noise into the data indicated that the fitting procedure was robust (Supplementary Figure S1). As expected, rates toward the LacI-bound side of each nucleosome were reduced in the presence of LacI. Interestingly, in combination with the slower rate toward the LacO sites, rates away from the LacI-bound sides were increased. This rate increase was 5- or 6-fold for LacO(−11R) (C→B) and LacO(−11L) (C→D), respectively (Figure 6F, Supplementary Figure S2). If this rate increase upon the addition of LacI were an essential feature of the model, we would expect that preventing an increase in these rates would result in a poor fit to the data. To test this, we fixed the C→B rate of LacO(−11R) and C→D rate of LacO(−11L) to the values observed in the absence of LacI, and refit the plus-LacI data. With these single rate constraints, the resulting models failed to match the data (Supplementary Figure S3). As shown in Figure 6E, the closest fitting models with these rate constraints were unable to recover both the sharp decrease in starting material ('C') and the transient build-up of intermediates on the side opposite the LacO site ('B' for LacO(−11R) and 'D' for LacO(−11L), respectively). These results indicate that an essential characteristic of the LacI barrier for Chd1 is not only slowing down sliding toward the LacO-bound site, but also increasing the rate at which Chd1 shifts nucleosomes in the opposite direction.

DISCUSSION

Chd1 is an organizing chromatin remodeler that both assembles and redistributes nucleosomes into evenly spaced arrays (19–22). Using LacI as a model system, we show here how Chd1 responds to a protein bound to nucleosomal DNA. Our experiments reveal that Chd1 sliding activity is affected by LacI on both entry and exit DNA, which has important implications for responding to DNA-bound factors as well as nucleosome spacing.

On 0N80 nucleosomes, LacI limited the extent of repositioning by Chd1, showing that proteins bound to the nucleosome edge pose a barrier for sliding (Figure 1). While slowing the rate of sliding toward LacI, this barrier effect was not caused by an absolute block in Chd1 action, as nucleosomes were similarly shifted toward LacI for three different locations of LacO. Instead, the limited sliding correlated with exposure of 10–11 bp on the opposite side, suggesting that the barrier resulted from preferential sliding in the opposite direction as long as approximately 10–11 bp of extranucleosomal DNA was available on the other side (Figure 2). Although slower sliding toward LacI would be expected to create significant asymmetry in sliding rates, we also discovered that the rate of sliding away from LacI was increased 5- to 6-fold (Figure 6). These results suggest that the barrier effect of LacI arises from changes in sliding rates that redirect the population of nucleosomes away from bound LacO sites (Figure 7).

The idea that sliding rates bias the distribution of nucleosomes builds on a kinetic discrimination model used to describe centering of mononucleosomes on short DNA fragments by the ISWI remodeler ACF (13). In the kinetic discrimination model, faster sliding toward the side of the nucleosome with longer extranucleosomal DNA biases redistribution of nucleosomes away from DNA ends. An important difference from the model proposed for ISWI, however, is that here we find that sliding in one direction depends on both sides of the nucleosome. That is, for a given direction of sliding, if LacI is present on entry DNA, sliding slows down, whereas if LacI is bound to exit DNA, the rate of sliding increases. This sensitivity to exit DNA is distinct from previous models of remodeler regulation, which focused on entry-side DNA (13,46). This response to both sides of the nucleosome amplifies the asymmetry in the rates of nucleosome sliding more than would be possible with a rate change on only one side.

How might Chd1 respond to a protein on either entry or exit DNA of the nucleosome? Although the nucleosome has 2-fold symmetry, entry and exit DNA have distinct positions and orientations with respect to each SHL2 position, where the ATPase motor acts. Considering also the opposite responses in sliding rates—decreased rate with LacI on entry DNA and increased rate on exit DNA—we expect that

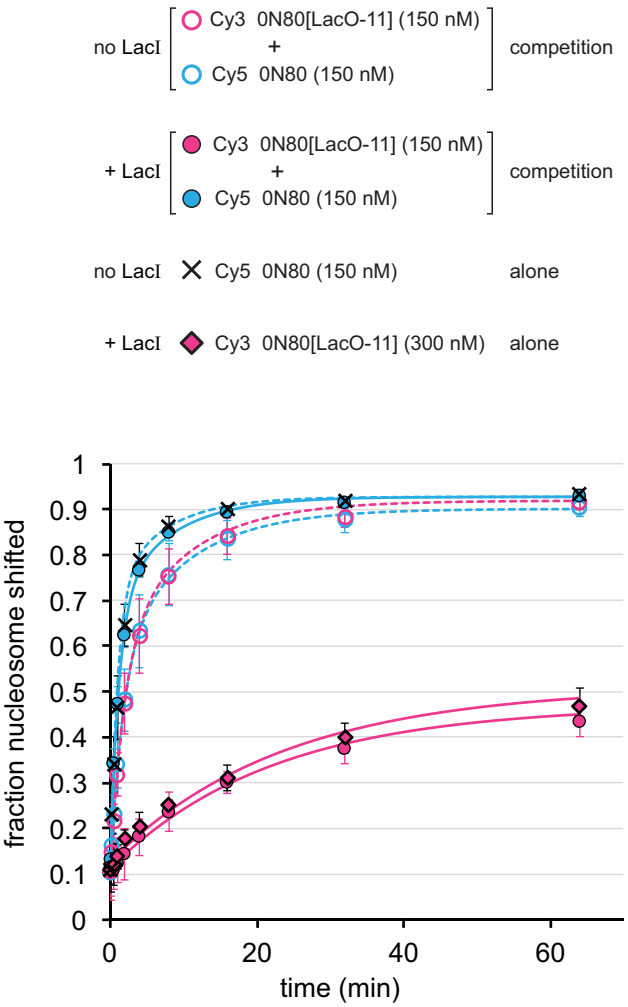

Figure 5. LacI does not sequester Chd1 on the nucleosome. Shown are Chd1 sliding experiments carried out with one or two types of nucleosome, in the presence (filled symbols, solid lines) or absence (open circles and 'X', dotted lines) of LacI. Nucleosomes lacking the LacO sequence were Cy5 labeled (blue in the graph), whereas nucleosomes containing the LacO sequence were Cy3 labeled (pink). Data were quantified from native gel sliding experiments, with each point showing the mean from six to eight independent experiments and error bars representing the standard deviations.

changes in Chd1 sliding activity most likely arise from two distinct mechanisms. Exactly how Chd1 activity is modulated is not presently clear, but our data suggest that one mechanism relies on sensing DNA flanking the nucleosome, whereas the other is independent from DNA availability outside the nucleosome core.

Despite the importance of the Chd1 DNA-binding domain for directing nucleosome sliding based on DNA outside the nucleosome core (15), here we found that the presence of LacI altered the pattern of nucleosome sliding by Chd1 even when the DNA-binding domain was deleted (Figure 4A). The lack of reliance of the barrier on extranucleosomal DNA was further supported by experiments showing that disruption of the bridge segment, required for responding to extranucleosomal DNA (Figure 4B), also did not relieve interference from LacI at the nucleosome edge (Figure 4C). Since the response to LacI on the nucleosome did not depend on sensing extranucleosomal DNA, Chd1 must also be sensitive to another signature of a factor bound to nucleosomal DNA.

We believe that such a signature likely results from perturbation of histone-DNA interactions. For experiments where one side of the nucleosome lacked extranucleosomal DNA, Chd1 shifted part or all of the LacI-bound site onto the nucleosome (Figures 1 and 2). Competition between histones and bound transcription factors has been well documented, and transcription factor binding on entry/exit DNA can stimulate DNA unwrapping of nucleosomal DNA (28,47). Given the slower sliding toward LacO-bound sites, which still acted as barriers after being completely shifted onto the nucleosome, we consider it likely that such unwrapping may be a primary means for sensing entry-side DNA of the nucleosome.

In contrast, the faster sliding associated with movement away from a LacI-bound site is consistent with sensing DNA outside the nucleosome core. A basic characteristic of Chd1 is its ability to 'center' mononucleosomes, where end-positioned nucleosomes are preferentially shifted toward the center of a DNA fragment (14,15). While this biased directionality has been considered to stem from activation by long flanking DNA on one side, the data presented here suggests that sliding away from DNA ends is also stimulated by the absence of available DNA on the exit side of the nucleosome. LacI was found to block available extranucleosomal DNA with the LacO(−11) site on 0N11 nucleosomes (Figure 3), and this position of LacI increased the rate that Chd1 shifted nucleosomes in the opposite direction (Figure 6). Therefore, the more rapid sliding away from LacI could explain the bias for sliding mononucleosomes away from DNA ends. Importantly, the increased sliding rate away from the LacI-bound site implies an autoinhibitory mechanism for Chd1, where the available flanking DNA on the exit side of the nucleosome reduces the rate of sliding. Such a mechanism would allow a change in DNA availability on the exit side to induce asymmetry in sliding rates, biasing nucleosomes to be repositioned away from the side where DNA is not available.

The sensitivity of Chd1 to both entry and exit sides of the nucleosome has important consequences for nucleosome positioning and stability of DNA-bound factors. Nucleosomes compete with transcription factors, not only through making binding sites less accessible, but also by directly stimulating dissociation of proteins bound to nucleosomal DNA (48). Disruption of transcription factor binding, for example, was shown to result from repositioning nucleosomes onto occupied binding sites by the SWI/SNF remodeler (49). Thus, a more rapid response of Chd1 to shift nucleosomes away from proteins on exit DNA increases the likelihood that these factors will remain bound to their sites. In addition to a faster and tighter response, the sensitivity of Chd1 to both sides of the nucleosome also allows for a wider range of remodelling outcomes. As shown by experiments using end-positioned nucleosomes where sliding away from LacI was hampered by lack of extranucleosomal DNA, Chd1 was capable of shifting the LacO site completely onto nucleosomal DNA. This ability to continue shifting a nucleosome toward a DNA-bound protein im-

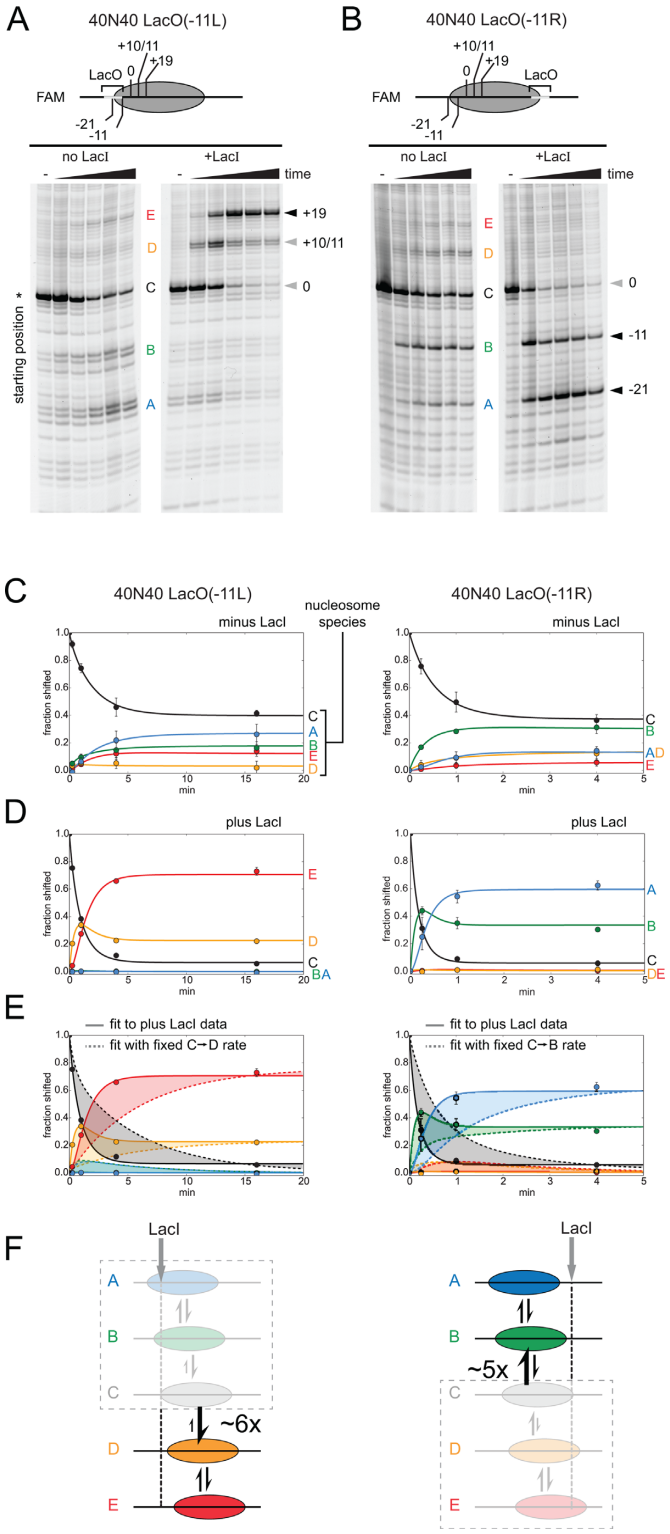

Figure 6. Chd1 shifts nucleosomes more rapidly away from Lac repressor. Centered 40N40 nucleosomes (150 nM) with a LacO site on the left (**A**) or right (**B**) were repositioned by Chd1 (50 nM) in the presence and absence of LacI. Nucleosome positions are shown by histone mapping at position H2B(S53C), with DNA fragments separated on DNA sequencing gels. Time points were 0, 0.25, 1, 4, 16, 64 min. Fits to a five state kinetic model for the quantified 40N40 sliding experiments are shown in the absence (**C**) and presence (**D**) of LacI. (**E**) Faster nucleosome sliding away from the LacO sites in the presence of LacI is required for fitting the kinetic model to the data. Fits for the plus-LacI data were carried out with the rate away from LacO (C→D for LacO(−11L) and C→B for LacO(−11R)) fixed to the slower values obtained in the absence of LacI. The dotted lines show the best fits from >800 000 trials (see Supplementary Figure S3 for the best 1000 trials), all of which fail to match the data. The colouring between the curves emphasizes the differences in fits with and without this rate constraint. (**F**) Summary of the kinetic models. For 40N40 nucleosomes with LacO on the left or the right, the relative rates of repositioning away from the LacO site was increased whereas shifting toward LacO was decreased compared with the rates obtained for the absence of LacI. States that are disfavoured in the presence of LacI due to asymmetry in forward/reverse rates are partially hidden by dotted boxes. Error bars in (C–E) indicate standard deviations from three independent mapping experiments for each condition. See also Supplementary Figure S1 and S2.

plies that Chd1 also has the potential to increase dynamics of transcription factor binding, depending on the chromatin environment.

In addition to using DNA-bound proteins such as transcription factors to guide nucleosome positioning, the sensitivity of Chd1 to both entry and exit DNA also has implications for nucleosome stability and spacing. The ability to generate evenly-spaced nucleosome arrays (19–22,50) indicates that Chd1 also responds to neighboring nucleosomes. Previous work showed that when nucleosomes begin to overlap and occupy territories of their neighbors, unstable intermediates can be generated that likely arise from DNA unwrapping from the nucleosome edge (51). We propose that unwrapping due to closely packed or overlapping nucleosomes would stimulate a Chd1 response similar to the presence of LacI on entry/exit DNA. For nucleosomes beginning to be unwrapped by their neighbors, biased sliding in the opposite direction would therefore help stabilize histone octamers of each nucleosome. Moreover, analogous to the differences in sliding rates that allow a DNA-bound factor to act as a barrier, the asymmetry in sliding rates would be lost once the separation between nucleosomes allows for mutual rewrapping and sufficient exposure of intervening extranucleosomal DNA. We therefore propose that the responses of Chd1 to a DNA-bound protein can explain its characteristic of generating evenly-spaced nucleosome arrays.

Finally, it is well established that Chd1 and ISWI share many biochemical characteristics *in vitro* and *in vivo*, and with this work we can now include the bias for sliding nucleosomes away from DNA-bound factors. Given their biochemical and architectural similarities, we hypothesize that ISWI remodelers also sense both entry and exit sides of the nucleosome, and therefore have an autoinhibitory mechanism for slowing down sliding in the presence of available extranucleosomal DNA on the exit side. Despite these common responses, it is becoming increasingly clear that Chd1 and ISWI remodelers play specialized roles in reorganization of chromatin *in vivo*: yeast Isw1 and Chd1 generate nucleosome arrays with longer or shorter repeats, respectively (50), whereas Isw2 positions 'founder nucleosomes' against which these arrays pack (52). We look forward to future work that reveals how basic characteristics like the ones described here have been adapted for specialized cellular roles.

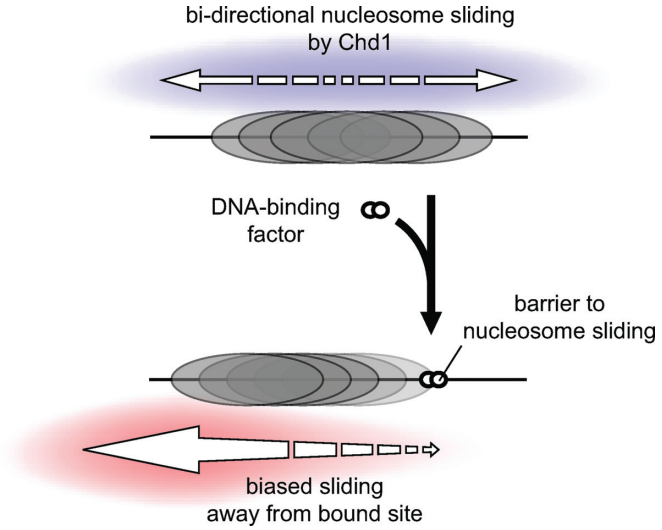

Figure 7. Sensing both sides of the nucleosome allows for greater asymmetry in nucleosome sliding rates. In the absence of DNA-bound factors, nucleosomes are dynamically shifted in either direction by chromatin remodelers such as Chd1. The association of a DNA-binding protein such as a transcription factor at the edge of a nucleosome decreases the rate of sliding toward and increases sliding away from the bound factor, which promotes rapid redistribution of nucleosomes away from the bound factor. By enforcing asymmetry in the bidirectional sliding of the nucleosome, DNA-binding proteins effectively provide barriers that direct nucleosome positioning.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

We thank Geeta Narlikar for discussions, sharing unpublished results, and the histone H4(A15C) expression plasmid, Mitch Lewis for the LacI expression plasmid, Li Ma and Guy Montelione for the Trigger Factor co-expression plasmid, and Sarah Woodson for discussions and generously sharing equipment.

FUNDING

National Institutes of Health (NIH) [R01GM-084192, R01GM-113240 to G.D.B., R01GM-059849 to M.D.W.]; National Science Foundation [MCB-0820293 to M.D.W.]. Funding for open access charge: NIH R01 [084192].
Conflict of interest statement. None declared.

REFERENCES

1. Simpson,R.T. (1990) Nucleosome positioning can affect the function of a cis-acting DNA element in vivo. *Nature*, **343**, 387–389.
2. Lomvardas,S. and Thanos,D. (2002) Modifying gene expression programs by altering core promoter chromatin architecture. *Cell*, **110**, 261–271.
3. Schones,D.E., Cui,K., Cuddapah,S., Roh,T.Y., Barski,A., Wang,Z., Wei,G. and Zhao,K. (2008) Dynamic regulation of nucleosome positioning in the human genome. *Cell*, **132**, 887–898.
4. Hartley,P.D. and Madhani,H.D. (2009) Mechanisms that specify promoter nucleosome location and identity. *Cell*, **137**, 445–458.
5. Radman-Livaja,M. and Rando,O.J. (2010) Nucleosome positioning: How is it established, and why does it matter? *Dev. Biol.*, **339**, 258–266.
6. Bai,L. and Morozov,A.V. (2010) Gene regulation by nucleosome positioning. *Trends Genet.*, **26**, 476–483.
7. Hughes,A.L. and Rando,O.J. (2014) Mechanisms underlying nucleosome positioning in vivo. *Annu. Rev. Biophys.*, **43**, 41–63.
8. Narlikar,G.J., Sundaramoorthy,R. and Owen-Hughes,T. (2013) Mechanisms and functions of ATP-dependent chromatin-remodeling enzymes. *Cell*, **154**, 490–503.
9. Bartholomew,B. (2014) Regulating the chromatin landscape: Structural and mechanistic perspectives. *Annu. Rev. Biochem.*, **83**, 671–696.
10. Flaus,A., Martin,D.M., Barton,G.J. and Owen-Hughes,T. (2006) Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. *Nucleic Acids Res.*, **34**, 2887–2905.
11. Hota,S.K. and Bartholomew,B. (2011) Diversity of operation in ATP-dependent chromatin remodelers. *Biochim. Biophys. Acta*, **1809**, 476–487.
12. Zofall,M., Persinger,J. and Bartholomew,B. (2004) Functional role of extranucleosomal DNA and the entry site of the nucleosome in chromatin remodeling by ISW2. *Mol. Cell. Biol.*, **24**, 10047–10057.
13. Yang,J.G., Madrid,T.S., Sevastopoulos,E. and Narlikar,G.J. (2006) The chromatin-remodeling enzyme ACF is an ATP-dependent DNA length sensor that regulates nucleosome spacing. *Nat. Struct. Mol. Biol.*, **13**, 1078–1083.
14. Stockdale,C., Flaus,A., Ferreira,H. and Owen-Hughes,T. (2006) Analysis of nucleosome repositioning by yeast ISWI and Chd1 chromatin remodeling complexes. *J. Biol. Chem.*, **281**, 16279–16288.
15. McKnight,J.N., Jenkins,K.R., Nodelman,I.M., Escobar,T. and Bowman,G.D. (2011) Extranucleosomal DNA binding directs nucleosome sliding by Chd1. *Mol. Cell. Biol.*, **31**, 4746–4759.
16. Bruno,M., Flaus,A., Stockdale,C., Rencurel,C., Ferreira,H. and Owen-Hughes,T. (2003) Histone H2A/H2B dimer exchange by ATP-dependent chromatin remodeling activities. *Mol. Cell*, **12**, 1599–1606.
17. Kassabov,S.R., Zhang,B., Persinger,J. and Bartholomew,B. (2003) SWI/SNF unwraps, slides, and rewraps the nucleosome. *Mol. Cell*, **11**, 391–403.
18. Tsukiyama,T., Palmer,J., Landel,C.C., Shiloach,J. and Wu,C. (1999) Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. *Genes Dev.*, **13**, 686–697.
19. Ito,T., Bulger,M., Pazin,M.J., Kobayashi,R. and Kadonaga,J.T. (1997) ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell*, **90**, 145–155.
20. Gkikopoulos,T., Schofield,P., Singh,V., Pinskaya,M., Mellor,J., Smolle,M., Workman,J.L., Barton,G.J. and Owen-Hughes,T. (2011) A role for Snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization. *Science*, **333**, 1758–1760.
21. Lusser,A., Urwin,D.L. and Kadonaga,J.T. (2005) Distinct activities of CHD1 and ACF in ATP-dependent chromatin assembly. *Nat. Struct. Mol. Biol.*, **12**, 160–166.
22. Pointner,J., Persson,J., Prasad,P., Norman-Axelsson,U., Stralfors,A., Khorosjutina,O., Krietenstein,N., Peter Svensson,J., Ekwall,K. and Korber,P. (2012) CHD1 remodelers regulate nucleosome spacing in vitro and align nucleosomal arrays over gene coding regions in *S. pombe*. *EMBO J.*, **31**, 4388–4403.
23. Yamada,K., Frouws,T.D., Angst,B., Fitzgerald,D.J., DeLuca,C., Schimmele,K., Sargent,D.F. and Richmond,T.J. (2011) Structure and mechanism of the chromatin remodelling factor ISW1a. *Nature*, **472**, 448–453.
24. Dang,W. and Bartholomew,B. (2007) Domain architecture of the catalytic subunit in the ISW2-nucleosome complex. *Mol. Cell. Biol.*, **27**, 8306–8317.
25. Hauk,G., McKnight,J.N., Nodelman,I.M. and Bowman,G.D. (2010) The chromodomains of the Chd1 chromatin remodeler regulate DNA access to the ATPase motor. *Mol. Cell*, **39**, 711–723.
26. Leonard,J.D. and Narlikar,G.J. (2015) A nucleotide-driven switch regulates flanking DNA length sensing by a dimeric chromatin remodeler. *Mol. Cell*, **57**, 850–859.
27. Clapier,C.R. and Cairns,B.R. (2012) Regulation of ISWI involves inhibitory modules antagonized by nucleosomal epitopes. *Nature*, **492**, 280–284.
28. North,J.A., Shimko,J.C., Javaid,S., Mooney,A.M., Shoffner,M.A., Rose,S.D., Bundschuh,R., Fishel,R., Ottesen,J.J. and Poirier,M.G.

(2012) Regulation of the nucleosome unwrapping rate controls DNA accessibility. *Nucleic Acids Res.*, **40**, 10215–10227.
29. Tsukiyama,T., Becker,P.B. and Wu,C. (1994) ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature*, **367**, 525–532.
30. Tsukiyama,T. and Wu,C. (1995) Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell*, **83**, 1011–1020.
31. Pazin,M.J., Bhargava,P., Geiduschek,E.P. and Kadonaga,J.T. (1997) Nucleosome mobility and the maintenance of nucleosome positioning. *Science*, **276**, 809–812.
32. Corona,D.F., Längst,G., Clapier,C.R., Bonte,E.J., Ferrari,S., Tamkun,J.W. and Becker,P.B. (1999) ISWI is an ATP-dependent nucleosome remodeling factor. *Mol. Cell*, **3**, 239–245.
33. Kang,J.G., Hamiche,A. and Wu,C. (2002) GAL4 directs nucleosome sliding induced by NURF. *EMBO J.*, **21**, 1406–1413.
34. Patel,A., McKnight,J.N., Genzor,P. and Bowman,G.D. (2011) Identification of residues in chromo-helicase-DNA-binding protein 1 (Chd1) required for coupling ATP hydrolysis to nucleosome sliding. *J. Biol. Chem.*, **286**, 43984–43993.
35. Nodelman,I.M. and Bowman,G.D. (2013) Nucleosome sliding by Chd1 does not require rigid coupling between DNA-binding and ATPase domains. *EMBO Rep.*, **14**, 1098–1103.
36. Bertagna,A., Toptygin,D., Brand,L. and Barrick,D. (2008) The effects of conformational heterogeneity on the binding of the notch intracellular domain to effector proteins: A case of biologically tuned disorder. *Biochem. Soc. Trans.*, **36**, 157–166.
37. Luger,K., Rechsteiner,T.J. and Richmond,T.J. (1999) Preparation of nucleosome core particle from recombinant histones. *Methods Enzymol.*, **304**, 3–19.
38. Daber,R., Stayrook,S., Rosenberg,A. and Lewis,M. (2007) Structural analysis of lac repressor bound to allosteric effectors. *J. Mol. Biol.*, **370**, 609–619.
39. Lewis,M., Chang,G., Horton,N.C., Kercher,M.A., Pace,H.C., Schumacher,M.A., Brennan,R.G. and Lu,P. (1996) Crystal structure of the lactose operon repressor and its complexes with DNA and inducer. *Science*, **271**, 1247–1254.
40. Lowary,P.T. and Widom,J. (1998) New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J. Mol. Biol.*, **276**, 19–42.
41. Kassabov,S.R. and Bartholomew,B. (2004) Site-directed histone-DNA contact mapping for analysis of nucleosome dynamics. *Methods Enzymol.*, **375**, 193–210.
42. Narlikar,G.J., Phelan,M.L. and Kingston,R.E. (2001) Generation and interconversion of multiple distinct nucleosomal states as a mechanism for catalyzing chromatin fluidity. *Mol. Cell*, **8**, 1219–1230.
43. Schwanbeck,R., Xiao,H. and Wu,C. (2004) Spatial contacts and nucleosome step movements induced by the NURF chromatin remodeling complex. *J. Biol. Chem.*, **279**, 39933–39941.
44. Saha,A., Wittmeyer,J. and Cairns,B.R. (2005) Chromatin remodeling through directional DNA translocation from an internal nucleosomal site. *Nat. Struct. Mol. Biol.*, **12**, 747–755.
45. Zofall,M., Persinger,J., Kassabov,S.R. and Bartholomew,B. (2006) Chromatin remodeling by ISW2 and SWI/SNF requires DNA translocation inside the nucleosome. *Nat. Struct. Mol. Biol.*, **13**, 339–346.
46. Hwang,W.L., Deindl,S., Harada,B.T. and Zhuang,X. (2014) Histone H4 tail mediates allosteric regulation of nucleosome remodelling by linker DNA. *Nature*, **512**, 213–217.
47. Li,G. and Widom,J. (2004) Nucleosomes facilitate their own invasion. *Nat. Struct. Mol. Biol.*, **11**, 763–769.
48. Luo,Y., North,J.A., Rose,S.D. and Poirier,M.G. (2014) Nucleosomes accelerate transcription factor dissociation. *Nucleic Acids Res.*, **42**, 3017–3027.
49. Li,M., Hada,A., Sen,P., Olufemi,L., Hall,M.A., Smith,B.Y., Forth,S., McKnight,J.N., Patel,A., Bowman,G.D. *et al.* (2015) Dynamic regulation of transcription factors by nucleosome remodeling. *Elife*, **4**, doi:10.7554/eLife.06249.
50. Ocampo,J., Chereji,R.V., Eriksson,P.R. and Clark,D.J. (2016) The ISW1 and CHD1 ATP-dependent chromatin remodelers compete to set nucleosome spacing in vivo. *Nucleic Acids Res.*, doi:10.1093/nar/gkw068.
51. Engeholm,M., de Jager,M., Flaus,A., Brenk,R., van Noort,J. and Owen-Hughes,T. (2009) Nucleosomes can invade DNA territories occupied by their neighbors. *Nat. Struct. Mol. Biol.*, **16**, 151–158.
52. McKnight,J.N., Tsukiyama,T. and Bowman,G.D. (2016) Sequence-targeted nucleosome sliding in vivo by a hybrid Chd1 chromatin remodeler. *Genome Res.*, **26**, 693–704.