

Regulation of DNA translocation of chromatin remodeler enzyme Chd1 by exit DNA unwrapping

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Abstract

Nucleosomes are the fundamental unit of chromatin. Chromatin remodeler plays a crucial role in the regulation of gene expression in eukaryotes. It is involved in important physiological processes, such as development, immune response, and metabolic regulation. During gene expression regulation, chromatin remodelers slide nucleosomes along genomic DNA and play a major role in chromatin organization. Chd1 senses the extranucleosomal linker DNA and controls nucleosome spacing in cells. However, the mechanism of linker DNA sensing by Chd1 is not completely understood. Here, we report the cryo-electron microscope (cryoEM) structures of Chd1 engaging nucleosomes in different states. Chd1 induces two exit-DNA conformations, either fully wrapped or partially unwrapped states. Notably, in the unwrapped conformation, the exit DNA interacts with a positively charged loop of the motor, named the exit-DNA binding loop, and traps Chd1 in the closed state in the ATPase cycle, suggesting attenuation of its remodeling activity. Explored single-molecule fluorescence resonance energy transfer (smFRET) and biochemical data supported the regulation of Chd1 remodeling activity by the exit-DNA conformations, which is important for the linker DNA sensitivity. Mutants of the Chd1 exit-DNA binding loop compromised nucleosome organization in yeast cells. Together, our findings provide valuable insights into Chd1 regulation by exit DNA unwrapping. These results provide a new perspective for the study of cell development and metabolism.

Keywords: chromatin remodeling; Chd1; nucleosome spacing; chromatin organization; gene expression regulation

Introduction

The nucleosome, the fundamental structural unit of eukaryotic chromatin, is composed of a histone octamer wrapped around by ~147 bp DNA [1]. The deposition of nucleosomes along the genomic DNA is not random, but exhibits organized patterns, with regularly spaced nucleosomal arrays around the promoter regions of active genes and densely packed nucleosomes in heterochromatin regions [2, 3]. Distinct nucleosome “repeat lengths” exist in different tissues and species [4, 5]. Deregulation of nucleosome positioning and spacing is associated with increased intragenic transcription and altered gene expression [6–8].

Chromatin remodelers are the main determinants of chromatin organization in cells [9, 10]. They are ATP-dependent motor proteins that slide, eject, space, and recompose nucleosomes. In eukaryotes, most chromatin remodeling complexes are highly conserved. They are involved in the expression of many signaling

proteins. The function of the chromatin remodeling complex is generally thought to directly affect the gene structure itself. The action mechanism of step-wise DNA translocation underpinning various chromatin remodeling activities has recently been illustrated [11]. These enzymes contain two conserved RecA-like ATPase motor domains and undergo transitions between open and closed conformations during the ATPase cycle, driving the fundamental DNA translocation reaction [12]. To ensure the proper chromatin organization, the DNA translocation activity of the elemental motors is regulated by the auxiliary domains and/or subunits within a complex, and the regulatory mechanisms are of central interest in epigenetics [12, 13].

The chromatin remodelers Chd1 and ISW1 are the major nucleosome spacers in yeast, which promote the formation of nucleosome arrays with repeat lengths of ~160, and ~175 bp, respectively [14–17]. *In vitro*, Chd1 moves the nucleosome towards

the center of a short DNA fragment [17, 18]. An interesting characteristic of Chd1, called linker DNA sensing, is that its DNA-translocation activity is modulated by the length of the linker DNA. Linker DNA sensing by Chd1 depends on its DNA-binding domain (DBD), which tethers the enzyme to the DNA substrate and directs nucleosome sliding towards the long linker DNA (the entry DNA) [19–21]. In contrast, the interaction between the ATPase motor and the DBD bound to the exit DNA was associated with the inhibition of the ATPase activity of Chd1, suggesting a regulatory role of the exit DNA to the chromatin remodeling function of Chd1 [22]. In the absence of the nucleosome substrate, Chd1 activity is autoinhibited by its double chromodomains [18]. Recently, several structures of the Chd1-nucleosome complex were reported, which reveal that the DBD binds to the exit DNA and promotes DNA unwrapping [22–25]. However, the underlying mechanism of exit DNA inhibition of Chd1 activity is not completely understood.

To explore the Chd1 mechanism, we purified yeast Chd1-nucleosome complexes and determined their cryo-electron microscope (cryoEM) structures in ADP and ADP-BeF₃ states. We found that Chd1 induces two exit-DNA conformations, either fully wrapped or partially unwrapped states. Notably, in the unwrapped conformation, the exit DNA interacts with a positively charged loop of the motor, named exit-DNA binding loop, and traps Chd1 in the closed state in the ATPase cycle, suggesting attenuation of its remodeling activity. In addition, a positively charged loop mediates the interaction between Chd1 and exit DNA and facilitates the unwrapping of the latter. Moreover, biochemical and explored single-molecule fluorescence resonance energy transfer (smFRET) analyses confirmed that the mutations of the positively charged residues in the interacting loop weaken the inhibitory effect of the exit DNA on Chd1's nucleosome remodeling activity. In conclusion, our study offers valuable insights into the DNA translocation mechanism of Chd1 by sensing linker DNA.

Results

Two conformations of the exit DNA of the nucleosome bound by Chd1

Chd1 (residues 167–1274), including the double chromo domains (dCD), the motor domains (lobe1 and lobe2), and the C-terminal DBD from *Saccharomyces cerevisiae*, was used to form complexes with the nucleosome core particle with 20 bp linker DNA at the TA-step poor “weak side” of the asymmetric “601” sequence [26] (167-NCP, Supplementary Table S1). The samples were subjected to single-particle cryoEM analyses (Supplementary Fig. S1). In the presence of a stable ATP analogue (ADP-BeF₃), the samples revealed two nucleosome-binding modes, with the exit DNA in the unwrapped and wrapped conformations, respectively. In one conformation (Class A), the motor bound at the super helical location 2 (SHL2), the double chromo domains bound at the SHL1, and the linker DNA was bound by the DBD and unwrapped about two helical turns (Supplementary Figs. S2a and S3a), similar to the previous studies [23–25]. We also identified a class of particles (Class A') showing unwrapped DNA, but the position of the DBD was unclear (Supplementary Fig. S2b), which might detach from the DNA during sample preparation, or bind the DNA at variable positions. The motor of the Class A' complex displayed a conformation similar to that of the Class A complex (Supplementary Figs. S2e and S3b). Therefore, we focused on the Class A complex below.

Interestingly, we identified a subset of particles (Class B) that showed fully wrapped exit DNA and partially unwrapped entry

DNA (Fig. 1a), and the structure was determined here at a resolution of 7.2 Å, which resolved the helical structures of the protein (Supplementary Fig. S2c, f, and g). The conformation of Class B has not been found in the previous studies [23–25] and was identified as a small fraction of the particles in the current study (Supplementary Fig. S1). The linker DNA in Class B was not clearly detected, which probably located at the entry as weak EM density was found at this side, whereas no extra EM density was found at the exit side (Supplementary Fig. S2c). This orientation of the nucleosome is opposite to that of the more populated Class A/A', and consistent with the idea that the Chd1 motor preferentially binds to the internal SHL2 and unwraps the “weak side” from the nucleosome edge (the exit DNA) [22, 27].

We also found particles with two molecules of Chd1 bound to the same nucleosome (Class C), the structure of which was determined at a resolution of ~6.9 Å (Supplementary Figs. S2d and S3c). Both wrapped and unwrapped conformations were detected in this Class. Our finding was consistent with the results of negative stain EM [22], in which the linker DNA showed wrapped and unwrapped conformations. With the exit DNA in the wrapped conformation, the nucleosome interacts with Chd1 in a canonical manner, highly similar to those of Snf2 and ISWI (Fig. 1b; Supplementary Fig. S3e) [28, 29].

The unwrapped exit DNA interacts with lobe1 of the motor

Relative to the wrapped conformation, the unwrapped exit DNA showed a rotation of ~60°, and notably altered the interaction with lobe1 of ATPase motor (Fig. 1c). Specifically, a positively charged loop (476-RAKGKK-481) was closer to the unwrapped exit DNA (Fig. 1d; Supplementary Fig. S3d), suggesting a role in promoting the unwrapped conformation. This loop, referred to as exit-DNA-binding loop (EDBL) herein, is a unique feature of Chd1 in yeast [24, 27], whereas the conserved Arg467 and Arg499 were closer to the wrapped exit DNA.

To test the regulation of exit DNA unwrapping by the interaction with lobe1, we explored smFRET assays to detect the conformations of the linker DNA of the nucleosome. We labeled the DNA at the nucleosome edge with Cy5, and H2A K119C with Cy3 (Fig. 2a). The structure indicated that DNA unwrapping increased the Cy3-Cy5 distance from ~5 nm to ~10 nm, and thus decreased the FRET signals. To avoid environmental interference, we collected the data showing a decrease in Cy5 intensity and an increase in Cy3 intensity (Cy3-Cy5 anticorrelation) as the unwrapping events (Supplementary Fig. S4a). In support of the function of the EDBL in promoting exit DNA unwrapping, the smFRET data indicated that the R476E/K478E/K480E/K481E mutations (the 4E mutant) displayed a dramatic loss of DNA unwrapping (Fig. 2b and c). Less unwrapping was not due to the loss of binding affinity, as the mutant showed no obvious defects in nucleosome binding or nucleosome-dependent ATPase activities (Supplementary Fig. S5a and b). Together, the data support the idea that the EDBL of lobe1 promotes the unwrapped conformation.

Chd1 fails to induce DNA translocation in the ADP-bound, exit DNA unwrapped state

DNA unwrapping is uniquely detected in Chd1, but potentially a regulatory step in the regulation of the enzyme. To detect how exit DNA unwrapping may regulate Chd1, we determined the structure of the Chd1-NCP complex in the ADP-bound state (Fig. 3a). Most of the complex formed with the “601” nucleosome in the ADP-bound state did not yield stable particles for the cryoEM analysis. We pursued a different substrate, the MMTV-A

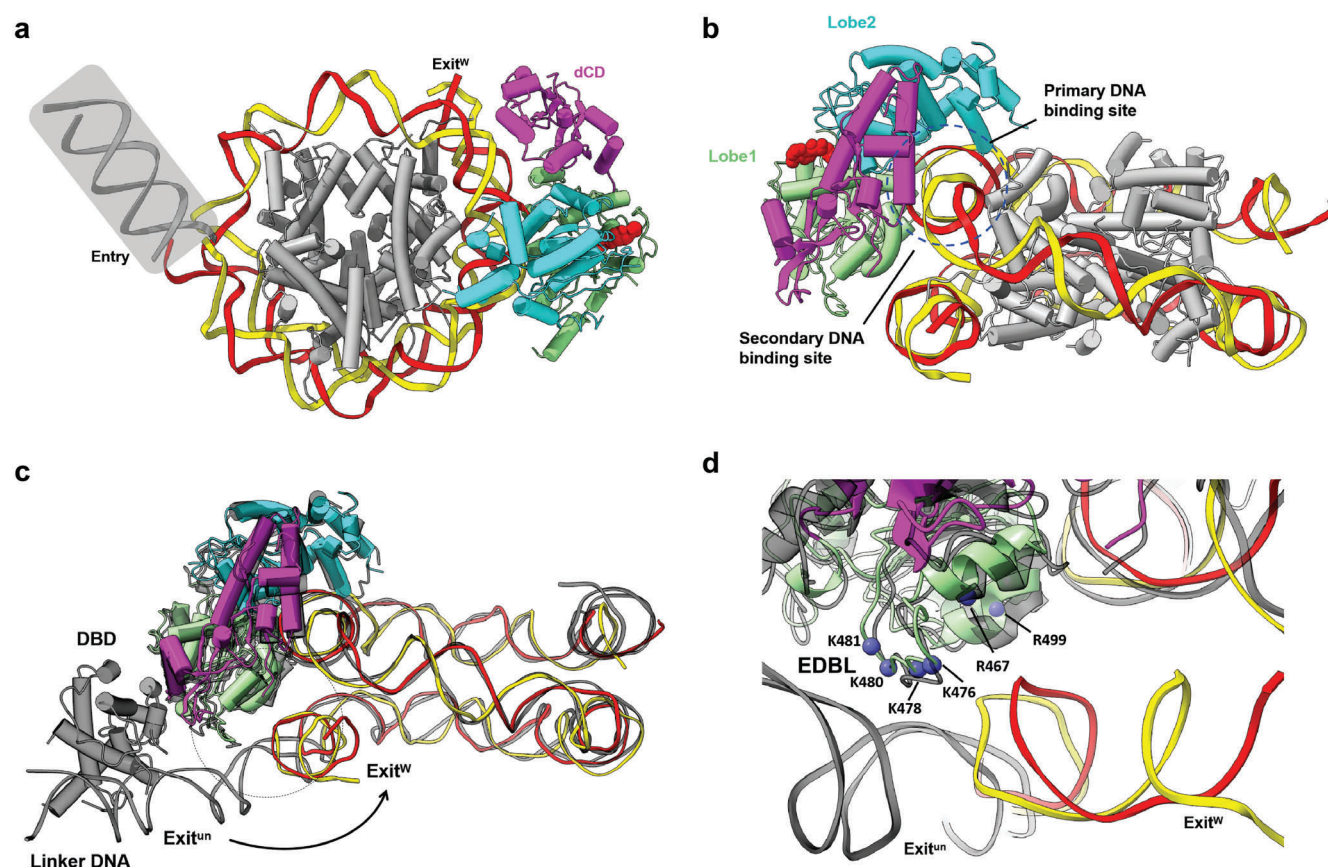


Figure 1 Structures of the Chd1-nucleosome complex in the presence of ADP-BeF₃. (a and b) Two views of the structure of the Chd1-nucleosome complex showing wrapped exit DNA (Exit^w). (c) Structural comparison of the Chd1-nucleosome complex with the exit DNA in the wrapped (color coded) and unwrapped (color gray) conformations. (d) The encircled region in (c) is enlarged for further analysis.

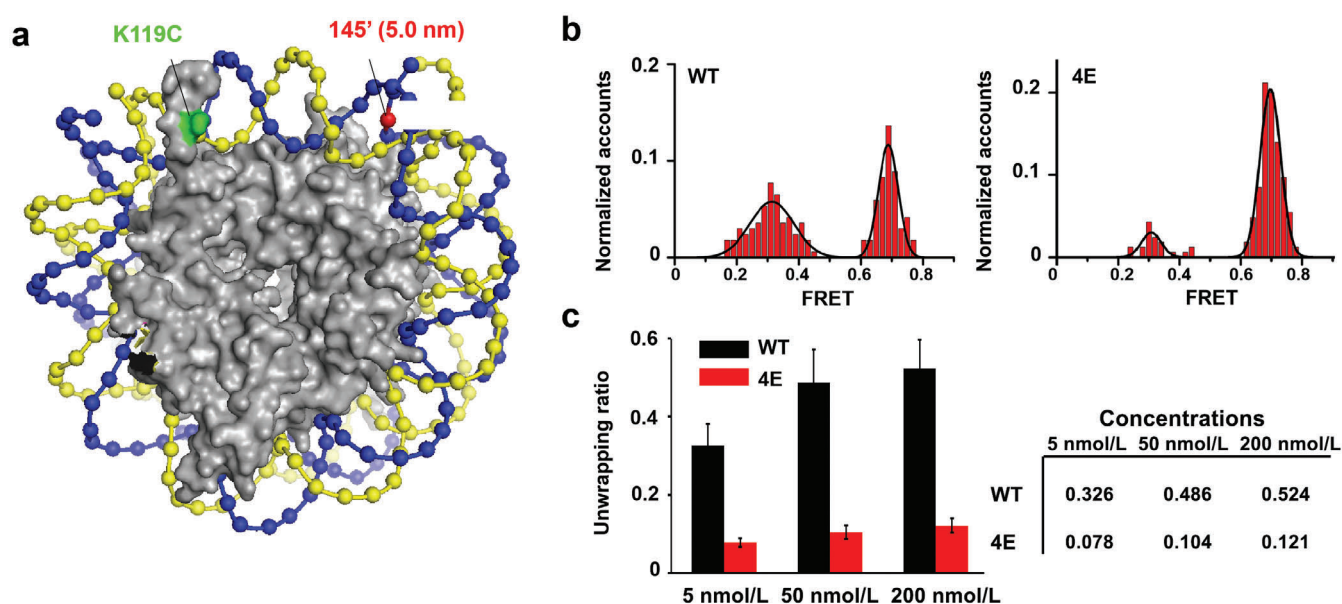


Figure 2 EDBL promotes the unwrapped conformation of the exit DNA. (a) Nucleosome labeling schemes. Red, Cy5; green, Cy3. The FRET pair distance is ~5 nm in the wrapped state. (b) Cy3-Cy5 FRET distributions of the nucleosomes bound by 200 nmol/L WT and 4E mutant Chd1 in the presence of ADP-BeF₃. The histograms were built from 169 (WT), and 165 (4E) traces, and fit with Gaussian distributions. (c) Quantification of the ratios of the unwrapping peaks at the indicated concentrations of WT and 4E mutant Chd1.

nucleosome. This nucleosome forms stable particles with Snf2, and the DNA is distorted in the same way as that of the “601” sequence [11]. A Stable complex of Chd1 bound to the MMTV-A

nucleosome in the presence of ADP was obtained, and the structure was refined to 3.9 Å (Supplementary Fig. S6). The Chd1 motor in the presence of ADP also induced exit DNA unwrapping

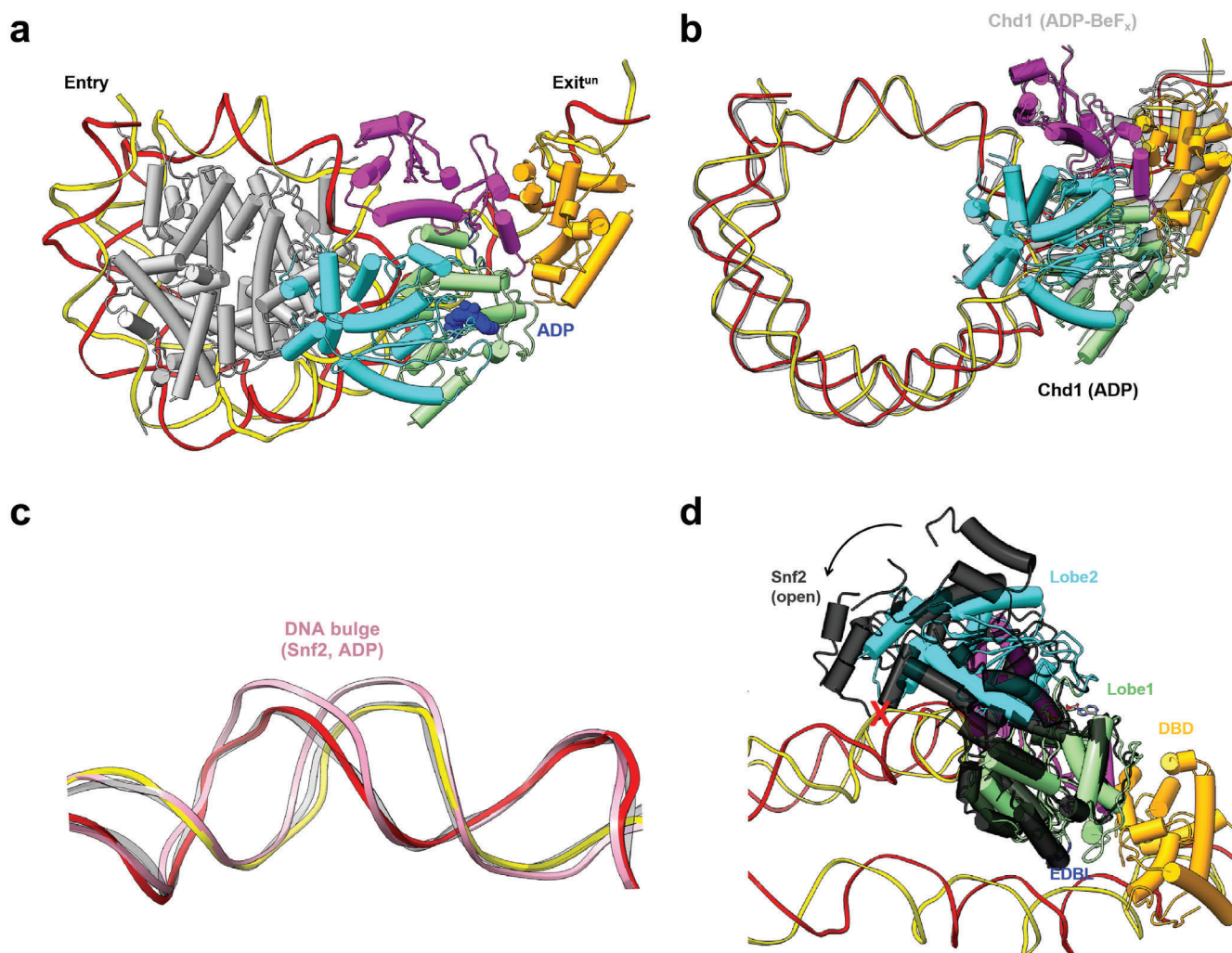


Figure 3 Structure of the Chd1-nucleosome complex in the presence of ADP. (a) Structure of the Chd1-nucleosome complex in the ADP-bound, exit DNA unwrapped state. (b and c) Structural comparisons of the Chd1-nucleosome complex in the ADP-bound (color coded) and ADP-BeF_x-bound (colored gray), exit DNA unwrapped states. The histone cores were aligned and omitted for clarity. DNA around the enzyme-binding site was shown in (c), superimposed with that bound by Snf2 (ADP, PDB code 6IY3) in pink [11]. (d) Structural comparison of Chd1 (closed, ADP, color coded) and Snf2 (open, ADP, colored gray). The structures of lobe1 are aligned. Arrow, proposed conformational change of lobe2 of Chd1 assuming the open state as that in Snf2. Red cross (X) indicates the clash between lobe2 and the DNA strand.

(Supplementary Fig. S7), and the bound ADP was clearly detected (Supplementary Fig. S7d). The unwrapped exit DNA interacted with lobe1 as that found in the ADP-BeF_x-bound state (Fig. 3b).

Interestingly, the structure showed that Chd1 in the ADP-bound, exit DNA unwrapped state did not open the primary DNA-binding cleft, and instead was locked in a closed conformation as that found in the ADP-BeF_x-bound state (Fig. 3b). This is in sharp contrast with Snf2 and ISWI, in which the motor domains undergo coordinated movements in different nucleotide-bound states, with lobe2 moving ~13° away from the dyad and lobe1 tilting ~7° upward away from the secondary DNA gyre (Supplementary Fig. S8), upon ATP hydrolysis and phosphate release [11, 29]. As a result of the closed conformation, Chd1 failed to induce the expected DNA bulge at the site of binding (Fig. 3c). This ADP-bound, closed state appears to go against the fundamental mechanism of DNA translocation [12], suggesting that Chd1 was in an inactivated state. Our structure is consistent with the recent biochemical analyses suggesting a partially disengaged state of Chd1 on the nucleosome in the ADP-bound state [30].

Coupling nucleosome sliding and linker sensing through the lobe1-exit DNA interaction

One prominent feature of Chd1 different from Snf2 and ISWI is the interaction with the unwrapped exit DNA. To examine how exit DNA unwrapping regulates Chd1, we compared the structure of Chd1 in the ADP-bound, closed state with the structure of Snf2 in the ADP-bound, open state. In keeping the position of lobe1 and the interaction with the unwrapped DNA, should Chd1 adopt the open conformation as that found in Snf2, the rotation of lobe2 would then clash with the DNA strand (Fig. 3d). Therefore, the inactivated structure of Chd1 suggests that the interactions between the unwrapped exit DNA and lobe1 inhibit the DNA translocation activity of the motor.

To validate this model of regulation by exit DNA, we tested the remodeling activity of the EDBL 4E mutant, which reduced exit DNA unwrapping, and should lead to a higher rate of DNA translocation. We employed a restriction site exposure (RSE) assay, in which the restriction site was placed near the left edge of the nucleosome, so that nucleosome sliding to the right (relative to the DNA track) resulted in RSE and digestion (Fig. 4a and b),

whereas nucleosome sliding to the left led to further occlusion and could not be detected in this assay. The linker DNA (80 bp) was placed at either side of the nucleosomes, 80N0 and 0N80, respectively.

Wild-type (WT) Chd1 showed a low, but reproducible sliding activity (nucleosome sliding to the right) towards the 80N0 nucleosome, resulting in ~5% of nucleosomal DNA cutting in 16 min (Fig. 4a). The low activity was probably because of the presence of the inhibitory exit linker DNA, the lack of entry linker DNA, and the asymmetric nature of the “601” nucleosome [31]. Importantly, compared with the WT enzyme, the 4E mutant showed 15% cutting of the 80N0 nucleosome in 16 min (Fig. 4a). Fitting the data indicated a ~5-fold increase of the initial reaction rate of the 4E mutant, consistent with the model that the 4E mutant was released from the exit DNA inhibition.

The activation by the 4E mutations is specific towards the substrate with exit DNA. With the more favorable, exit-DNA-free 0N80 nucleosome as the substrate, the mutant showed a lower activity compared to the WT enzyme (Fig. 4b). The reduced activity was possibly due to a general defect by the introduction of negative charges that interfered with the interaction with the nearby secondary DNA gyre [28]. The data support our model that

interaction between lobe1 and the unwrapped exit DNA inhibits DNA translocation.

The nucleosome sliding assays above indicated that the 4E mutant does not show a general defect in ATP-dependent translocation but gains an activity towards the 80N0 nucleosome, and loses an activity towards the 0N80 nucleosome, which suggests dysregulation of linker sensing of the enzyme. To further test the defect in linker DNA sensing, we employed a nucleosome centering activity, in which Chd1 sensed the linker DNA and moved the initially end-positioned nucleosomes towards more central positions (Fig. 4c). As expected, WT Chd1 showed a robust centering activity, whereas the 4E mutant markedly reduced the centering activity to a level ~8% of the WT protein. Similar conclusion was made using a 4A mutant EDBL (Supplementary Fig. S5c). Together, the data revealed that the loss of the nucleosome centering caused by the EDBL mutations was not because of the less remodeling efficiency but uncoupling linker DNA sensing from nucleosome translocation.

Inhibition of DNA translocation by exit DNA unwrapping detected by smFRET

The finding that DNA translocation is coupled to the conformations of the exit DNA is unexpected. To detect the conformation

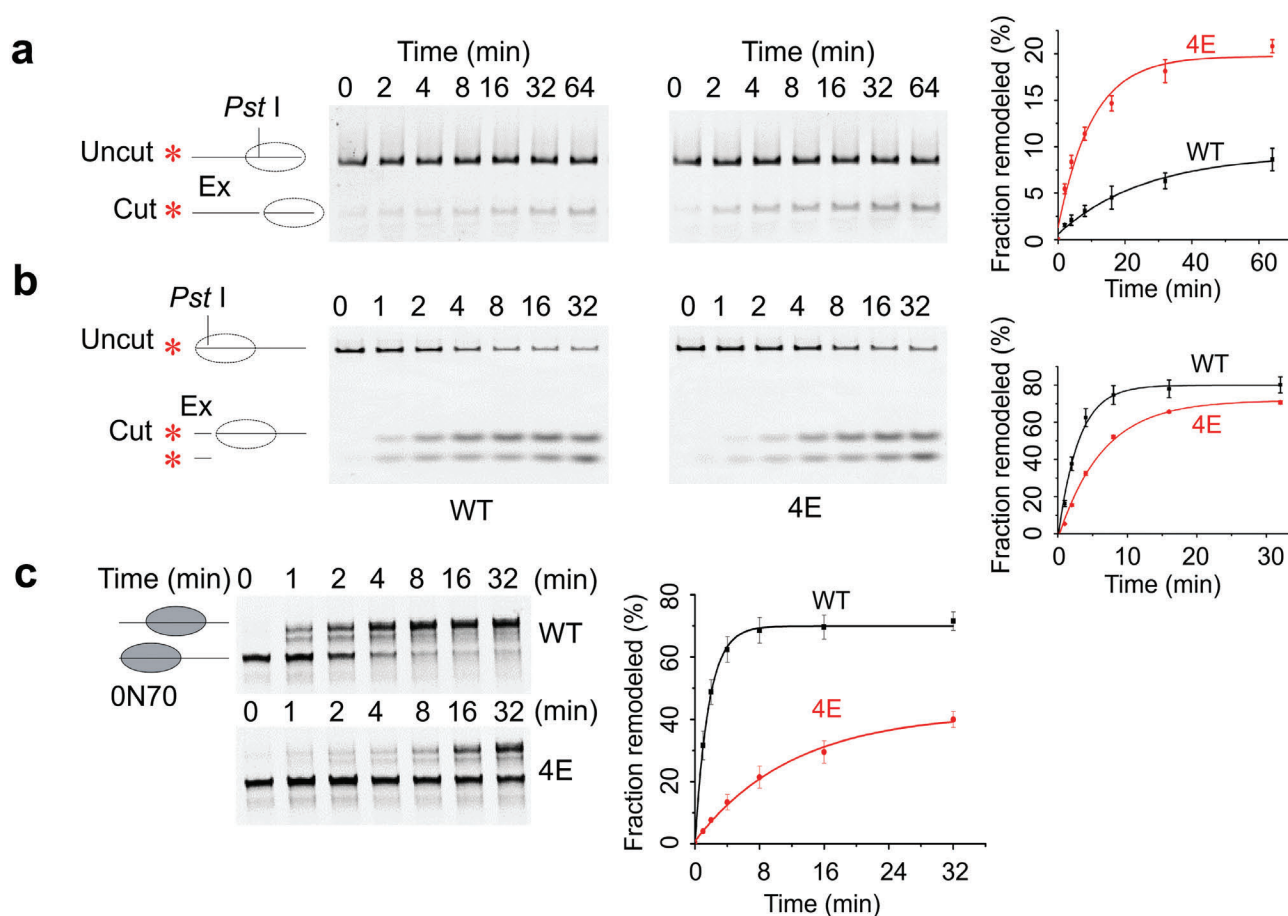


Figure 4 Chromatin remodeling activities of WT and 4E mutant of Chd1. (a) Nucleosome sliding activities of WT and 4E mutant as showed by the RSE assays using the 80N0 nucleosomes. Representative gels are shown on the left, and quantification of the fractions remodeled is shown on the right. The initial remodeling rates of 4E and WT were estimated to be 0.018 and 0.0033 min⁻¹ towards the 80N0 nucleosome, respectively. Ex, exit DNA. Error bars indicate SD of the means ($n = 3$ independent experiments). (b) Nucleosome sliding activities of WT and 4E mutant towards the 0N80 nucleosomes. The initial remodeling rates of 4E and WT were estimated to be 0.12 and 0.28 min⁻¹ towards the 0N80 nucleosome, respectively. Error bars indicate SD of the means ($n = 3$ independent experiments). (c) Nucleosome centering activities of WT and 4E mutant. Quantification of remodeled products is shown on the right. The initial remodeling rates were estimated to be 0.033 and 0.41 min⁻¹ for the 4E and WT enzymes, respectively. Error bars indicate SD of the means ($n = 3$ independent experiments).

of the exit DNA and DNA translocation simultaneously, we developed a three-color smFRET assay (Fig. 5a and b). The phosphate backbone of the nucleosome was labeled with Cy5 at an internal site (Supplementary Fig. S4b and Supplementary Table S1), and the other side with the 30 bp linker DNA was labeled with Alexa488, and histone H2A (K119C) was labeled with Cy3. As discussed above, the Chd1 motor preferentially binds to the “strong side” of the asymmetric “601” nucleosome, and unwraps the “weak side” exit DNA. Therefore, we referred to the side with 30 bp linker DNA (weak side, Supplementary Table S1) as the exit DNA (otherwise as specified). Exit DNA unwrapping increases the Alexa488-Cy3 distance and thus decreases the Alexa488-Cy3 FRET value. Translocation of the tracking strand of the entry DNA (the side with 40 bp linker DNA) towards the dyad is expected to increase the Cy3-Cy5 distance, and hence decrease the FRET value. We alternatively excited the samples with 488 nm and 532 nm lasers, and calculated the Alexa488-Cy3 and Cy3-Cy5 FRET values to detect DNA unwrapping and DNA translocation,

respectively (Supplementary Fig. S4c and d). The samples were heterogeneous, and to maximize the dynamic range for smFRET measurements, the nucleosomes with a single Cy3 on the proximal H2A were selected for further analysis (Supplementary Fig. S4e).

After fluorescence intensity correction (Supplementary Fig. S9) [32], the unbound nucleosome displayed Alexa488-Cy3 and Cy3-Cy5 FRET peak values of 0.60 and 0.69, respectively (Supplementary Fig. S4c and d), respectively, indicating a fully wrapped structure of the nucleosome in the absence of Chd1. We added Chd1 in the presence of ADP. A fraction of the nucleosomes were induced to unwrap their exit DNA, as indicated by the decrease of the Alexa488-Cy3 FRET value to ~0.29 (Fig. 5c). Remarkably, these nucleosomes displayed no DNA translocation, as indicated by the little change of the Cy3-Cy5 FRET distribution. A fraction of nucleosomes with fully wrapped exit DNA displayed a new low Cy3-Cy5 FRET peak at ~0.62 (Fig. 5d). As indicated by the FRET peak values of the Cy5 probe placed at different

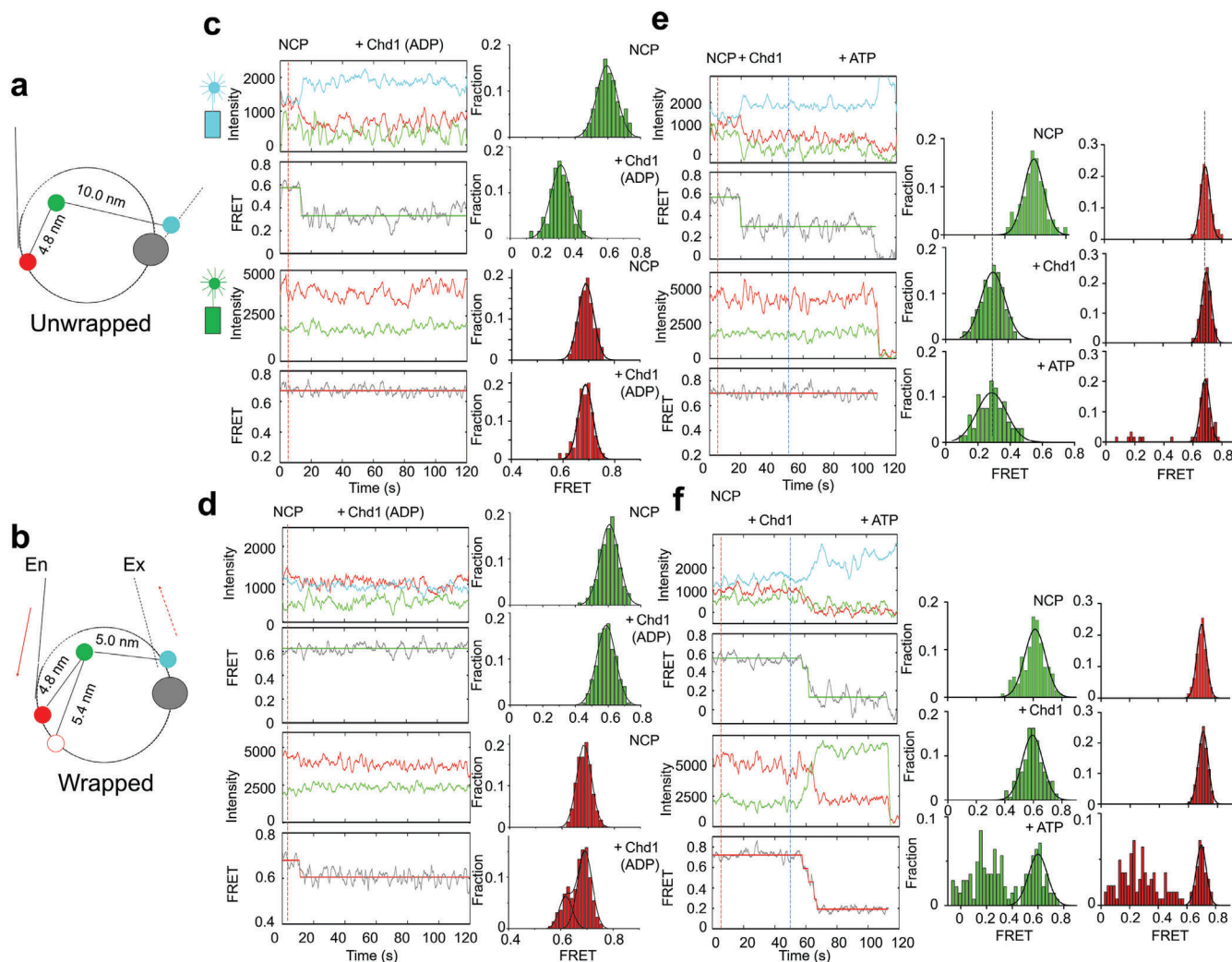


Figure 5 Three-color smFRET analyses of DNA translocation induced by Chd1. (a and b) Nucleosome labeling schemes. The samples are classified into two states based on the Alexa488-Cy3 FRET signals, unwrapped (a) and wrapped (b). Grey, Chd1; red, Cy5; green, Cy3; cyan, Alexa488; En, entry; Ex, exit. Arrows indicate the directions of DNA translocation. (c and d) Representative fluorescence (color-coded) and FRET (black) trace curves (left), and Alexa488-Cy3 (green) and Cy3-Cy5 (red) FRET distributions (right). Chd1 (ADP bound) was added to the nucleosomes (NCP), and data were sorted based on the unwrapped (c) and wrapped conformations (d) of the exit DNA. The samples were excited with alternating 488 nm and 532 nm lasers, schematically illustrated as cyan and green lights, respectively. (e and f) Experiments were performed similarly as in (c and d) with Chd1 (ADP-BeF₃) bound and ATP added sequentially. The histograms were built from 65, 225, 63, and 142 traces in Fig. 5a, 5b, 5c, and 5d, respectively, and fit with Gaussian distributions.

positions of the nucleosome (Supplementary Fig. S4f), the change of the FRET peaks from 0.69 to 0.62 is consistent with DNA translocation with the elemental step size of 1 nucleotide register [11, 33, 34]. The unchanged Cy3-Cy5 FRET peak (Fig. 5d) was likely due to the unbound nucleosomes or enzyme binding at the opposite DNA gyre, either of which did not change the Cy3-Cy5 distance. Together, these data support that Chd1 induces canonical DNA translocation in the ADP-bound, exit DNA wrapped state, but it is reluctant to do so when the exit DNA is unwrapped.

We then performed the smFRET assays in the presence of continuous ATP hydrolysis, which provided further validation of the model in the ATPase cycles. To obtain a stable, initially unwrapped state, we first loaded the nucleosomes with Chd1 (ADP-BeF_x bound), and then infused ATP into the system. Because of bidirectional DNA translocation [35], some trace curves showed repetitive movement after the initial repositioning events (Supplementary Fig. S4g). So, we focused the analyses on the initial events, which showed directional DNA translocation. In support of our model, the nucleosomes induced by Chd1 to unwrap the exit DNA (reduced Alexa488-Cy3 FRET values) showed weak DNA translocation in the presence of ATP, as indicated by the majority of the Cy3-Cy5 FRET values remaining at ~0.69 (Fig. 5e). We noticed that a few cases (8 out of 63 unwrapped samples) showed decreased Cy3-Cy5 FRET values with unwrapped exit DNA (Supplementary Fig. S4h), suggesting that some samples have chances to escape the exit-DNA inhibition. The lack of DNA translocation was not caused by inhibition from the bound ADP-BeF_x, because the ATPase and remodeling assays indicated little inhibition under the conditions used (10 μ mol/L ADP-BeF_x and 3 mmol/L ATP; Supplementary Fig. S10). In contrast, the enzyme showed a robust translocation activity towards the nucleosome with the exit DNA in the wrapped conformation, as indicated by the large amount of samples showing low Cy3-Cy5 FRET values (Fig. 5f). The data support that exit DNA unwrapping diminishes the translocation efficiency.

A small fraction of the samples showing initial wrapped DNA conformation increased the Cy3-Cy5 FRET values after ATP was added (Supplementary Fig. S4e), which was consistent with the Chd1 motor bound at the opposite side of the nucleosome (the TA-step poor DNA gyre) and translocated the DNA to the reverse direction (Supplementary Fig. S4i). The data suggest a low population of Chd1 motor binding to the TA-step poor DNA gyre, consistent with the cryoEM observation (Supplementary Fig. S1).

Together, the three-color smFRET data in the presence of ADP and ATP support the idea that whereas the nucleosome undergoes typical DNA translocation with exit DNA in the wrapped conformation, exit DNA unwrapping antagonizes the DNA movement. Therefore, it is not the exit DNA *per se*, but the unwrapped conformation that inhibits DNA translocation.

The EDBL of Chd1 regulates nucleosome spacing *in vivo*

The biochemical data above indicate that the 4E mutant has a reduced, but not completely lost, sensing activity to the linker DNA. This is not unexpected, as the 4E mutations disrupt the inhibitory mechanism of exit DNA, but the enzyme still has the intact DBD, which is able to sense the entry linker DNA and promotes translocation [36]. To investigate the importance of the EDBL of Chd1 in nucleosome spacing *in vivo*, we determined the genome-wide nucleosome positioning of the yeast cells using MNase-seq. Deletion of both Chd1 and ISW1 in the yeast cells caused a dramatic loss of nucleosome spacing, suggesting that

they are the major remodelers to set the nucleosome organization [14]. Because Chd1 deletion alone caused modest changes [16, 37], we analyzed the Chd1 mutation in the genetic background of Δ ISW1. Consistent with the previous studies [14, 16], deletion of both ISW1 and Chd1 (Δ ISW1/ Δ Chd1) disrupted nucleosome spacing, and the repeated length could not be accurately determined (Fig. 6a). ISW1 deletion (Δ ISW1) reduced nucleosome repeated length from 165 bp to 162 bp. Consistent with the role of Chd1 in promoting a shorter linker-DNA length [16], disruption of the Chd1 activity by the EDBL mutation in the Δ ISW1 cells (Δ ISW1/4E) increased the global nucleosome-nucleosome distance from 162 bp to 167 bp. The 4E mutations did not perturb the median positions of the +1 nucleosomes much, but induced genome-wide shifts of the +2 to +5 nucleosomes by 4–9 bp (Fig. 6b; the statistical significance in Fig. 6c). These results were exemplified at several loci (Supplementary Fig. S11). In addition to the shifts of nucleosome positioning, the 4E mutations also reduced the nucleosome phasing, as indicated by the broadening of the peaking distributions. Based on a previous nucleosome phasing model [16], which describes the degree of order of the nucleosomal arrays, WT cells have the best phasing (14.7 ± 0.2 bp). Introducing 4E mutations to the Δ ISW1 mutant further weakened the global nucleosome phasing from 17 ± 0.2 bp to 19.6 ± 0.1 bp. Moreover, we detected the function of EDBL inside the yeast cells. Under the stressed conditions, in the presence of the genotoxic agent methyl methanesulfonate (MMS) at 37°C, the mutant cells displayed slow growth phenotype (Fig. 6d), suggesting defects in the expression of stress response genes. The 4E mutant conferred sensitivity to DNA damage. Together, the data support that exit DNA sensing by the EDBL of Chd1 regulates nucleosome organization in cells.

Discussion

In this work, we determined the cryoEM structures of Chd1 engaging the nucleosome in different states. In particular, we found that exit DNA unwrapping antagonizes the open conformation of Chd1 in the ADP-bound state. Biochemistry, smFRET, and yeast genetic analyses support regulation of the DNA translocation activity by the exit-DNA conformations.

Based on these findings, we propose a model of Chd1 regulation (Fig. 7). When the exit DNA is wrapped, Chd1 undergoes the canonical remodeling reactions in a manner essentially identical to those of Snf2 and ISWI [11, 29]. The smFRET data indicated that Chd1 translocated 1 bp DNA from the entry side in the presence of ADP, supporting an unified mechanism of DNA translocation [12]. As more linker DNA emerges from the exit side of the nucleosome, the DBD binds to the exit DNA ~10 bp at the linker region, and stabilizes the unwrapped conformation. The unwrapped exit DNA interacts with the EDBL of the motor. This interaction in turn antagonizes lobe1 from tilting up in the ADP-bound state, and inhibits the open conformation of the enzyme. The binding of the EDBL to the unwrapped exit DNA provides the mechanism to relay the availability of exit linker DNA to the motor, working as a brake to inhibit DNA translocation. The exit DNA-mediated brake mechanism seems not be tight, as indicated by the slight escape of exit DNA inhibition observed by the smFRET (Fig. 5e). Nevertheless, this regulation mechanism is important for the optimal Chd1 activity *in vitro* (Fig. 4), and for nucleosome spacing and phasing in cells (Fig. 6).

Although evenly spaced nucleosome arrays, which contain some DNA at the either side of the nucleosomes, are generally

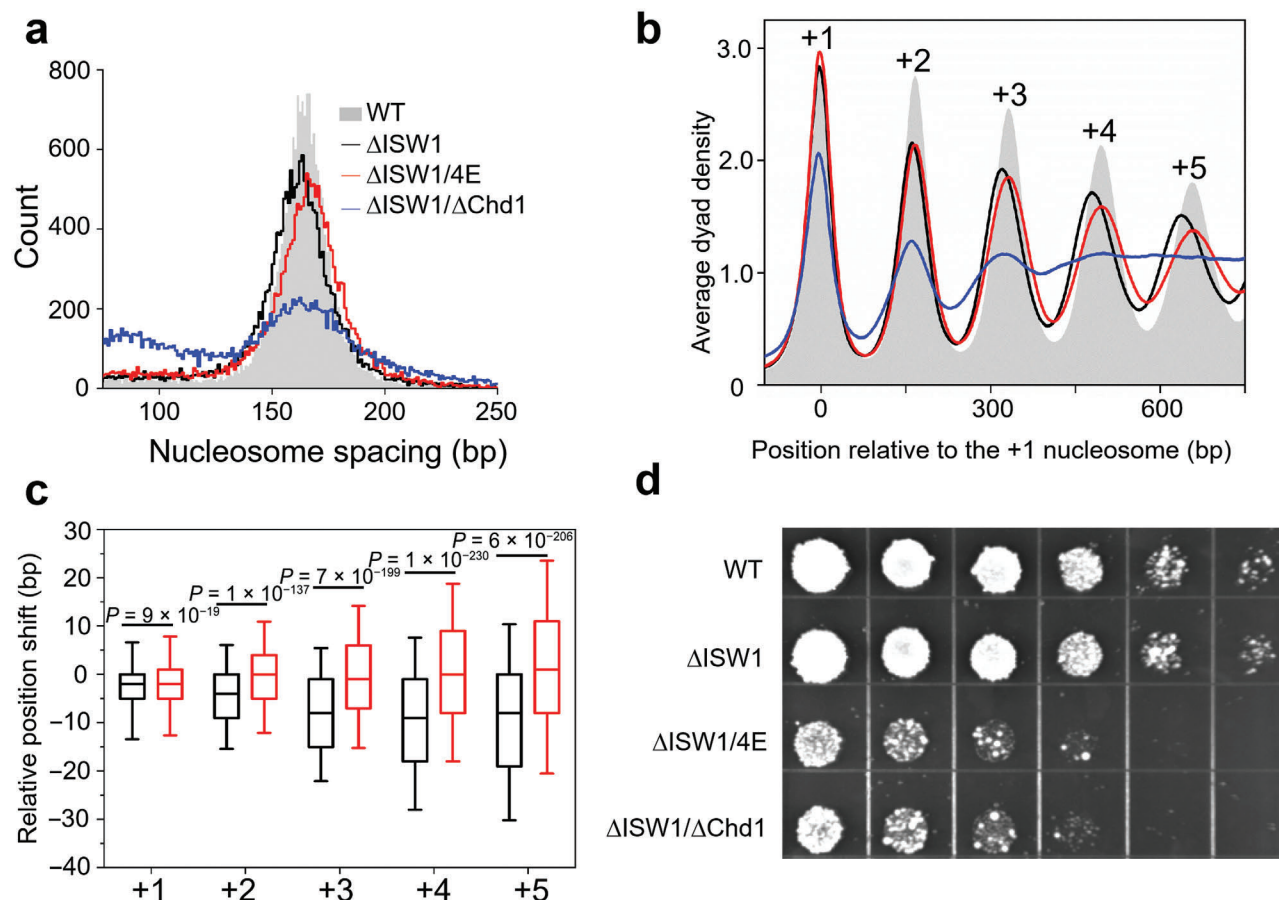


Figure 6 Exit DNA sensing by Chd1 regulates nucleosome spacing in yeast cells. (a) Altered nucleosome spacing distributions in the mutants. (b) Genome-wide nucleosome phasing profiles aligned on the dyad of the +1 nucleosome in WT and mutant cells (color coded in a). (c) Position shifts of the promoter proximal nucleosomes in the ΔISW1 (black) and ΔISW1/4E (red) mutants. The positions in WT cells were used as the reference. Box = 25%–75%, Middle line = median, Whiskers = SD, $n = 4570$ genes (ΔISW1), $n = 4518$ genes (ΔISW1/4E). Statistical significance was analyzed with the Wilcoxon-Mann-Whitney test. * $P < 10^{-10}$; ** $P < 10^{-100}$; *** $P < 10^{-200}$. (d) Growth assays of the WT and mutant yeast cells. All assays were performed in triplicate, and the representative ones are shown.

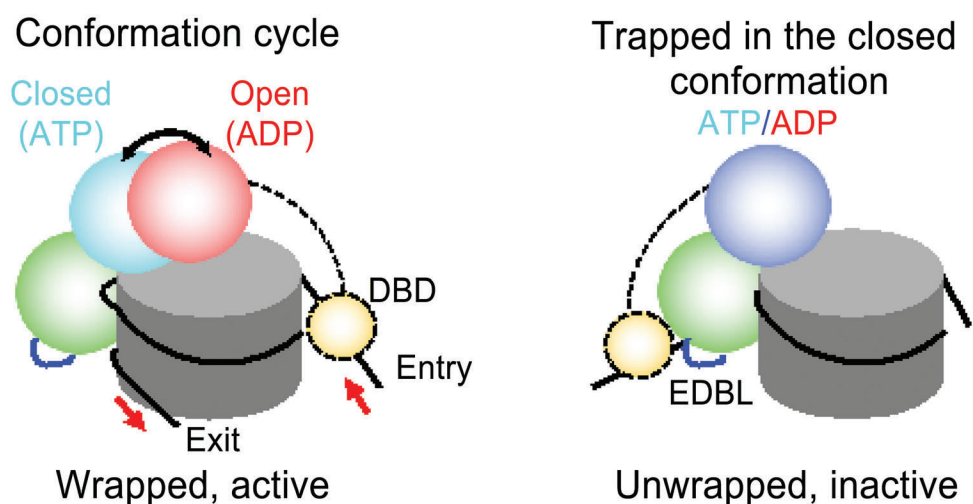


Figure 7 Regulation of nucleosome remodeling by exit DNA unwrapping. Schematic model of linker DNA sensing by Chd1 are shown. Arrow, direction of DNA translocation. With the exit DNA in the wrapped state, the Chd1 motor undergoes open-closed conformation cycles, translating DNA in the remodeling reaction. Binding of the DBD to the exit DNA (~10 bp) stabilizes the unwrapped conformation. The unwrapped exit DNA interacts with the EDBL and in turn stabilizes the motor in the closed conformation, which provides a brake mechanism to actively stop the remodeling activity.

observed in WT cells, closely packed dinucleosomes with no or very short linker DNA widely appear in the absence of Chd1 and ISW1 [37]. Our model of Chd1 regulation provides a mechanism

to resolve the closely packed dinucleosomes, and is consistent with the activity of Chd1 in forming nucleosome arrays with spacing of ~160 bp [38]. Chd1 is well known to sense the entry

DNA through its DBD domain to promote nucleosome sliding [36]. The exit DNA-EDBL interaction explains the notion of exit side inhibition [22, 39].

Various chromatin remodelers share common ATPase motor domains, but are regulated by distinct signals to ensure proper chromatin landscape in cells. In a remodeling cycle, DNA is partially translocated into the nucleosome by a remodeler in the ADP-bound state, which is a metastable intermediate associated with DNA distortion [12]. Our model of Chd1 regulation offers an example of how the auxiliary elements modulate the intermediate state to control the DNA translocation activity in response to the nucleosomal cue. Acting on the ADP-bound state, a pivot point in the translocation reaction, is conceivably a general, sensitive way to control the remodeling activity, and is different from the autoinhibition mechanisms occurring before engaging with the nucleosome substrate [18, 40, 41]. A new study reported an open conformation of Chd1 bound to the nucleosome in the unwrapped state, in which the nucleosome was tailored to stabilize the binding [42]. This is consistent with our observation that the exit-DNA inhibition is not tight. In agreement with our study, they showed that EDBL mutations induced a reduction of the centering activity. The quantitative fold changes of the reduction are different, which are probably due to the different experimental conditions.

Limitations of the study

This study focuses on how exit DNA unwrapping regulates DNA translocation of Chd1. However, the role of the sequence of exit DNA and other parts of nucleosomal DNA was not considered. The DNA translocation activity of chromatin remodelers, including Chd1, highly depends on the sequence of DNA. Therefore, whether the effect of exit DNA is sequence-specific is a problem worth studying. We will try to address this problem in further studies.

Materials and methods

Protein expression and purification

The gene of Chd1 (residues 167–1274) from *S. cerevisiae* genomic DNA was cloned and inserted into a modified pGEX-4T-2 (GST-tag) vector, in which the thrombin recognition site was replaced with tobacco etch virus (TEV) protease recognition site. The mutants were generated by Quickchange mutagenesis. All constructs were confirmed by DNA sequencing. The proteins were over-expressed in the *Escherichia coli* expression strain Rosetta (DE3) and cells were grown in LB media to an absorbance at 600 nm ($A_{600\text{ nm}}$) = 0.8 at 37°C, then induced with 0.5 mmol/L isopropyl- β -D-thiogalactoside (IPTG) at 18°C overnight.

Cells were spun down at a speed of 4,000 rpm (Beckman, Rotor JA4.2) for 15 min, and lysed by High Pressure Homogenizer Machine (ATS) at 4°C, in 50 mmol/L Tris-HCl, pH 8.0, 1 mol/L NaCl, 2 mmol/L dithiothreitol (DTT), and 1 mmol/L PMSF. The cell lysate was cleared by centrifugation at a speed of 18,000 rpm (Beckman, Rotor JA20) for 2 h, and the supernatant was separated and then loaded onto a gravity column with GST beads. After elution by 25 mmol/L reduced glutathione (GSH), the GST-tagged fusion protein was cleaved by incubating with ~0.02 mg/mL TEV protease overnight at 4°C. The proteins were further purified by an ion-exchange column (Source-15Q, GE Healthcare) with Tris buffer, pH 8.0, and then subjected to gel-filtration chromatography (Superdex-200, GE Healthcare) in buffer containing 20 mmol/L

Tris, pH 8.0, 150 mmol/L NaCl, 5 mmol/L DTT, and 5% glycerol. The purified protein was concentrated to ~10 mg/mL and stored at –80°C.

Nucleosome reconstitution

The nucleosomes were constituted with the “601” DNA positioning sequence, MMTV-A sequence (Supplementary Table S1), and *Xenopus laevis* histones as described previously [11].

ATPase, RSE, and nucleosome centering assays

Measurement of ATP hydrolysis was performed as previously described [43], using Chd1 (50 nmol/L) and 167-NCP (0.2–0.5 μ mol/L). The reactions led to substrate depletion at the very end of the reactions (Supplementary Fig. S5b). The ATPase activities were calculated at the early time points (< 1000 s) when the yield of product increased linearly.

The RSE assays using Cy5-labeled nucleosome 100N100 (5 nmol/L) and Chd1 (5 nmol/L) were performed as described [43], in which ATP was added at the end to initiate the reaction. In the one-side RSE assays, 40 nmol/L ON80 and 80N0 nucleosomes, and Chd1 at 4 nmol/L were used. Nucleosome centering assays were performed basically as described [41] with 40 nmol/L ON60 nucleosome and 4 nmol/L Chd1 at 25°C. Band intensities were quantified in Quantity One software, and the reaction rate constants were fit to a single exponential decay using Origin9.2.

Nucleosome binding assays

We assessed the nucleosome binding affinities through EMSAs as described [43]. 167-NCP (10 nmol/L) were mixed with increasing amounts of proteins in 20 mmol/L Tris-HCl, pH 7.5, 50 mmol/L KCl, 5 mmol/L $MgCl_2$, 5% glycerol, and 0.1 mg/mL bovine serum albumin. The apparent K_d values were estimated from the protein concentration at 50% saturation.

Yeast genetics

The yeast strain BY4741 (MATa *leu2 ura3 his3 met15 can1*) was used as WT, transformed with lithium acetate method. To generate the Δ ISW1 strain, the ISW1 gene was first replaced by a DNA fragment carried a KaMX gene, which enabled the cells to acquire the ability to grow on the G418 medium (YPD + G418). On the background of Δ ISW1, Chd1 was replaced by URA3 and the yeast cells were selected using SC/-Ura sodium medium to construct the Δ ISW1/ Δ Chd1 strain. To construct the Δ ISW1/4E strain, the URA3 gene was then replaced by Chd1^{4E} (R476E/K478E/K480E/K481E), and the cells were selected using 5-FOA sodium medium (SC + 5FOA). All the mutants were confirmed by PCR and DNA sequencing.

To determine the effect of mutations on yeast growth under stress conditions, we conducted a spot assay. All strains were cultured in YPD for 24 h to the platform stage. They were then diluted in YPD to the optical density (OD_{600}) of 0.1, and further cultured at 30°C until the OD_{600} reached 1. The cotransformants were spotted on drug-containing plates (YPD + 0.02% MMS) and incubated at 30°C for 3 days for drug sensitivity testing to assess their sensitivities. All assays were performed in triplicate.

MNase-seq sample preparation and data analysis

Single colony of each strain was selected and cultured in YPD at 30°C overnight in two biological replicates. We diluted the yeast cells at 1:200 and cultured for 9 h. Cells were harvested and converted to spheroplasts using 0.25 mg/mL Zymolase100T (MP Biomedicals cat#320932). Spheroplasts were digested with

Micrococcal Nuclease (NEB M0247S) at concentrations of 600, 1200, and 2400 gel unit/mL, respectively. Samples were treated with Proteinase K (25 µg/mL, Tiangen RT403) for 30 min at 65°C to remove protein. Phenol:chloroform was used to extract DNA. We incubated the sample with RNase A (100 µg/mL, Tiangen RT405) to remove RNA. We detected the nucleosomal DNA using 1.5% agarose gels and selected the proper samples with ~80% mononucleosome band for DNA library preparation using NEBNext Ultra II DNA Library Prep Kit (NEB #E7645S) [44]. Libraries were sequenced by NovaSeq 6000 System.

Pair-end sequencing reads were trimmed by Trimmomatic [45] and mapped to sacCer3 reference genome using Bowtie2 [46]. Reads in the range of 120–180 bp were selected, and those mapped to repetitive rRNA locus (chrXII: 451275–469084) were excluded [47]. SAMtools was used to remove possible PCR duplicates [11]. Nucleosome positions were identified using the algorithm as described in GeneTrack [48]. Briefly, nucleosome midpoint coordinates were smoothed by Gaussian filtering with a standard deviation of 20 bp. The peak of the smoothed curve defines the nucleosome position. The +1 nucleosome was defined as the nucleosome that was closest to and within ± 150 bp of TSS. A total of ~4700 genes larger than 750 bp and with at least five well-defined nucleosome positions (interval < 250 bp) were considered for downstream analysis [49, 50]. Nucleosome phasing was estimated based on the model proposed before [16, 51]. The three parameters of the model were fitted to +1 to +6 nucleosomes on the average dyad density profile. For nucleosome shift analysis, dyad position of WT and mutants was compared and the median shift was calculated for each +1 to +5 nucleosome [52]. For nucleosome spacing analysis, intervals between +1 to +5 nucleosomes were taken into account. The spacing distribution was then subjected to Gaussian fitting, and the centroid of the Gaussian curve was defined as an average spacing.

Sample preparation and EM data collection

The Chd1(167–1274)-NCP complexes were obtained by mixing 10 µmol/L protein with 5 µmol/L 167-NCP. We purified and stabilized the complexes using the GraFix as described before [11]. For cryoEM sample preparation, a drop of 4 µL sample was added to a glow-discharged Quantifoil holey carbon grid (R1.2/1.3, 300 mesh). After waiting for 60 s, the grid was blotted for 4.5 s (under 100% humidity and 8°C) and plunged into liquid ethane cooled by liquid nitrogen using FEI Vitrobot IV.

CryoEM data processing

The samples at ADP-BeF_x state and ADP state were observed using a Titan Krios microscope (FEI Company) operated at 300 kV equipped with Cs corrector and Gatan GIF Quantum energy filter (slit width 20 eV). The micrographs were collected with AutoEMationII [53] at a defocus range from 1.7 to 3.5 µm. A total of 10020 micrographs of samples at ADP-BeF_x state were collected with FalconII camera at a nominal magnification of ×75,000 (the corresponding pixel size being 0.882 Å) with movie mode for 33 frames with 8 s exposure time. The total dose was ~50 e⁻/Å². A total of 9786 micrographs of samples at ADP state were recorded on Gatan K2 Summit camera under super resolution mode at a nominal magnification of ×105,000, the corresponding binned 2-fold pixel size of which was 1.091 Å. Each micrograph was dose-fractionated to 32 frames with 0.175 s exposure time per frame. With a dose rate of 8.2 counts per physical pixel per second, the total dose was ~51 e⁻/Å². Motion-corrected integrated images generated by MotionCor2 were used for further processing [54]. The defocus parameters were determined with Gctf program [55].

The reconstruction was performed with Relion1.4 [56], Relion2.0 [57], and Relion3.0. For ADP-BeF_x state dataset, a total of 2,170 thousand particles were automatically picked with deep-learning based home-made scripts (developed by Zichuan Lin in Xueming Li's laboratory). After particle sorting and three rounds of 2D classification, a total of 2,100 thousand particles were selected and subjected to the first round 3D classification, using the SHL2 conformer of Snf2-NCP as the initial reference model [28]. A total of 491290 Chd1-NCP particles were selected to subject to auto-refinement, resulting in a 3D reconstruction map at a 3.68 Å resolution. In order to obtain the Class A' and Class B particles, the second round of 3D classification was performed with local angular search step at 3.75 degree. A total of 312042 particles of the Class A' complex and 29276 particles of the Class B complex were selected and gave rise to a 3D reconstruction with overall resolutions of 3.39 Å and 7.19 Å, respectively, after auto-refinement. Another two rounds of 3D classification were performed to find the Class C particles, and eventually a total of 42714 particles were selected, which produced a 3D reconstruction at 6.93 Å after auto-refinement.

For ADP state dataset, a total of 1,590 thousand particles were automatically picked with deep learning based home-made scripts. Particle sorting and several rounds of 2D classification were performed to remove ice contaminated and broken particles. A total of 2,086 thousand particles were selected and subjected to the first round of 3D classification with the Class A map in the ADP-BeF_x bound state as the initial model. To obtain good classes, the SHL6 conformer of Snf2-NCP [28] was low-pass filtered to 15 Å to guide the second round of 3D classification. These particles were selected and performed the third round of 3D classification with the generated class map as a reference by itself. A total of 42503 particles of Chd1-NCP particles were selected and refined to the 4.4 Å resolution map. The selected 42,503 particles as seeds were then combined with each subset to purify more intact particles similar to the methods described before [58]. After seed-facilitated 3D classification, a total of 177,824 particles with clear features were combined for further processing. After 3D classification with local search, the best class was combined with the former selected 42,503 particles. The repeated particles were removed and the resulting 56,412 particles were refined to the 3.9 Å resolution map.

All the 3D classification was performed with 2x binning particles and auto-refinement with 1x binning particles. The resolution was estimated using the gold-standard Fourier shell correlation with 0.143 criterion [59].

Single-molecular FRET assay

Double-stranded (ds) DNA constructs were created by annealing and ligating a set of overlapping as described previously [11]. All nucleosomes for the smFRET experiments were constituted with the DNA fragment showed in Supplementary Table S1. We constructed a series of duplex-DNA sample with different Cy5 dye labeling sites, which were A20, G21, G22, and C23, to further investigate the distance dependence of Cy3-Cy5 FRET pair (Supplementary Fig. S4f).

To give an example oligonucleotide sequences, the following oligonucleotides were used to assemble the dsDNA for the nucleosome with backbone Cy5 labeled into tracking strand G21 DNA phosphate backbone.

Tracking strand:

5'-GAGTTCATCCCTTATGTGATGGACCCTATACGCGGCCGCCCTGGAGAATCCCGGTGCCGA-3';

5'-/5Cy5/GGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCT
TAAACGCACGTACGCGCTGTCCCC-3';

5'-/5Phos/GCGTTTAAACGCCAAGGGGATTACTCCCTAGTCTCC
AGGCACGTGTCAGATATATACAT/5Alexa488/CCT-3';

5'-GAAGCTTGTCTGAGAAGTACTAGAGGATCATAA-3'.

Guide strand:

5'-/5Bioteq/TTATGATCCTCTAGTACTTCTCGACAAGCTTCAGGA
TGTATATATCTGACA-3';

5'-/5Phos/CGTGCCTGGAGACTAGGGAGTAATCCCCCTTGGCGGT
TAAACGCGGGGACACGCGTACGT-3';

5'-/5Phos/GCGTTTAAAGCGGTGCTAGAGCTGTCTACGACCAATT
GAGCGGCTCGGCACCGGATTCTCC-3';

5'-/5Phos/AGGCGGCGCGGTATAGGGTCCATCACATAAGGGAT
GAACTC-3'.

An analogous strategy was used to label Cy5 into the DNA at different positions. For site-specific Cy3 labeling of histone H2A, a cysteine substitution was introduced at residue 119 (K119C). A ratio of 1:1 of Cy3-labeled and unlabeled H2A mixture was used to reconstitute FRET-labeled nucleosomes. It generated three kinds of Cy3-labeled nucleosomes: (i) with Cy3 labeled to the H2A proximal to the Cy5 on the DNA, (ii) with Cy3 labeled to the H2A distal to the Cy5 on the DNA, and (iii) with both H2A labeled by Cy3. They were distinguished at the single-molecule level. To maximize the dynamic range for smFRET measurements, we selected the population with the highest FRET value (Cy3-Cy5 FRET pair), which corresponded to nucleosomes with a single Cy3 on the proximal H2A for further analysis.

The smFRET sample preparation and data analysis were performed similarly as described before [11]. To investigate the unwrapping efficiency, we reconstituted the 40N30 nucleosomes with Cy3-Cy5 FRET pair at the positions similar to those of the Cy3-Alexa488 pair. Subsequently, 5, 50, and 200 nmol/L WT and 4E Chd1 with 25 μ mol/L ADP-BeF_x were added to the nucleosomes, and the initial and the last FRET states were analyzed, respectively. Some cases showed a decrease of Cy5 intensity and no change in Cy3 intensity, which might result from Chd1 binding or environmental changes surrounding Cy5 rather than separation from the Cy3 donor (Supplementary Fig. S4a). We collected the data showing decrease in Cy5 and increase in Cy3 intensity as the unwrapping events.

Samples containing Alexa488 and Cy3 were alternatively excited by 488 nm and 532 nm lasers using a switching box reported before [60]. The signals of Alexa488, Cy3, and Cy5 channels were separated by a 532 nm and 630 nm dichroic mirror and detected by an EMCCD camera. The 40N30 nucleosomes at a concentration of 100 pmol/L were anchored to coated quartz slides. After a few seconds (~5 s) imaging, 200 nmol/L Chd1 with 0.5 mmol/L ADP was added. In the two-step reactions, 200 nmol/L Chd1 with 10 μ mol/L ADP-BeF_x was added to the nucleosomes and recorded about 50 s, and then 3 mmol/L ATP was added subsequently. The 14-bit movie with time resolution of 300 ms was recorded and analyzed.

In the three-color FRET experiments, an alternating laser excitation was achieved by switching the 488 nm and the 532 nm lasers using a switching box. The Alexa488-Cy5 FRET was barely detectable at long distances (>9 nm and > 12 nm in the wrapped and unwrapped states, respectively). The Cy3-Cy5 FRET value excited with the 532 nm laser was calculated as described before [11]. The Alexa488-Cy3 FRET value excited with the 488 nm laser was calculated as described [32]:

$$1 - \frac{I_{Alexa488}^{Cor}}{I_{Alexa488}^{Cor} + I_{Cy3}^{Cor} + I_{Cy5}^{Cor}}$$

$I_{Alexa488}^{Cor}$, I_{Cy3}^{Cor} , and I_{Cy5}^{Cor} are the fluorescence intensities of Alexa488, Cy3, and Cy5 upon excitation of 488 nm lasers, respectively. This FRET value reflects the distance between the linker DNA labeled with Alexa 488 and histone H2A labeled with Cy3, and the conformations of the linker DNA.

The intensities of $I_{Alexa488}^{Cor}$, I_{Cy3}^{Cor} , and I_{Cy5}^{Cor} were corrected as follows:

$$I_{Alexa488}^{int} = \frac{I_{Alexa488}^{raw}}{(1 - Alexa488_{leak})}$$

$$I_{Cy3}^{int} = \frac{I_{Cy3}^{raw} - I_{Alexa488}^{int} * Alexa488_{leak}}{(1 - Cy3_{leak})}$$

$$I_{Cy5}^{int} = \frac{I_{Cy5}^{raw} - I_{Cy3}^{int} * Cy3_{leak}}{Cy5_{eff}}$$

$$I_{Alexa488}^{cor} = \frac{I_{Alexa488}^{int}}{Alexa488_{eff}}$$

$$I_{total} = \frac{I_{Alexa488}^{cor} + I_{Cy3}^{int} + I_{Cy5}^{int}}{1 + Cy3Dir}$$

$$FRET_{Alexa488-Cy3} = 1 - \frac{I_{Alexa488}^{cor}}{I_{total}}$$

$$I_{Cy3}^{cor} = I_{Cy3}^{int} - Cy3Dir * (1 - FRET_{Cy3-Cy5}) * I_{total}$$

$$I_{Cy5}^{cor} = I_{Cy5}^{int} - Cy3Dir * FRET_{Cy3-Cy5} * I_{total}$$

$I_{Alexa488}^{raw}$, I_{Cy3}^{raw} , and I_{Cy5}^{raw} are the original intensities of Alexa488, Cy3, Cy5 upon excitation of 488 nm lasers, respectively. $I_{Alexa488}^{int}$, I_{Cy3}^{int} , and I_{Cy5}^{int} are the intermediate intensities of Alexa488, Cy3, Cy5 in calculation, respectively. $FRET_{Cy3-Cy5}$ is Cy3-Cy5 FRET value which is calculated by the data from excitation of 532 nm laser. The fluorescence intensities were corrected to account for the direct excitation of Cy3 ($Dir_{Cy3} = 0.26$), the difference in quantum yield and registration efficiency between Cy3 ($Cy3_{eff} = 1$), Cy5 ($Cy5_{eff} = 0.96$), and Alexa 488 ($Alexa488_{eff} = 2$), as well as for the bleed through from the Alexa488 into the Cy3 channel ($Alexa488_{leak} = 0.33$) and from Cy3 into the Cy5 channel ($Cy3_{leak} = 0.1$).

In the ADP-bound state, we divided the traces into two parts: before adding ADP (NCP, 0–5 s) and after adding Chd1-ADP (Chd1, after 5 s). We analyzed each part by the step-finding algorithm [11]. After adding Chd1-ADP, the Alexa488-Cy3 FRET signals were decreased in a fraction of the traces. We assigned these traces to the unwrapped state (Fig. 5a), and the histogram is shown in Fig. 5c (colored green). The histogram of the corresponding Cy3-Cy5 FRET values is also shown in Fig. 5c (colored red). In a fraction of the traces, the Alexa488-Cy3 FRET values remained unchanged. We assigned these traces to the wrapped state (Fig. 5b) and built the histogram in Fig. 5d (colored green). The histogram of the Cy3-Cy5 FRET values of these molecules were also built in Fig. 5d (colored red).

In the two-step assays, we divided the traces into three parts: before adding Chd1-ADP-BeF_x (NCP, 0–5 s), after adding Chd1-ADP-BeF_x but before adding ATP (+ Chd1, 5–50 s), and after adding ATP (+ATP, after 50 s). The data were analyzed similarly as above using the step-finding algorithm [11], and assigned to the unwrapped (Fig. 5e) and wrapped (Fig. 5f) states before adding ATP. Continuous DNA translocation occurred in a fraction of the samples after ATP addition, as indicated by the decreased Cy3-Cy5 FRET values. The Cy3-Cy5 FRET values of these traces were analyzed at the last step of the initial decrease, which may be

followed by periodic regain, and the corresponding Alexa488-Cy3 FRET values of the same molecules were also analyzed. Because of the scarcity of the unwrapped events in the two-step assays, 25 traces from the 40N30 NCP and 38 traces from the 20 nt-T 20N30 nucleosome (sequences in Supplementary Table S1) were combined for the analysis, and are showed in Fig. 5e.

Supplementary data

Supplementary material is available at *Life Metabolism* online.

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Conflict of interest

The authors declare that no conflict of interest exists.

Author contributions

Yuanyuan Tian (Conceptualization [Equal], Data curation [Equal], Funding acquisition [Equal], Investigation [Equal], Methodology [Equal], Supervision [Equal], Writing - original draft [Equal]), Qi Jia (Data curation [Equal], Investigation [Equal], Methodology [Equal]), Meijing Li (Data curation [Equal], Methodology [Supporting]), Youyang Sia (Investigation [Equal], Methodology [Equal]), Pengjing Hu (Methodology [Supporting]), Kangjing Chen (Methodology [Supporting]), Ming Li (Resources [Equal]), Xueming Li (Methodology [Supporting]), Zigang Xu (Resources [Equal], Funding acquisition [Equal]), Lin Ma (Resources [Equal]), Youpi Ye (Funding acquisition [Equal], Investigation [Equal], Methodology [Equal]), Ying Lu (Data curation [Equal], Investigation [Equal], Methodology [Equal]), and Zhucheng Chen (Conceptualization [Lead], Funding acquisition [Lead], Resources [Lead], Supervision [Lead], Writing - review & editing [Lead]).

Ethics approval

All procedures were performed in accordance with the Ethics Committee of Tsinghua University.

Data availability

All study data are included in the article and/or supplementary information. Materials are available upon request.

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