The fundamental unit of chromatin, the nucleosome, is an intricate structure that requires histone chaperones for assembly. ATAD2 AAA+ ATPases are a family of histone chaperones that regulate nucleosome density and chromatin dynamics. Here, we demonstrate that the fission yeast ATAD2 homolog, Abo1, deposits histone H3–H4 onto DNA in an ATP-hydrolysis-dependent manner by in vitro reconstitution and single-tethered DNA curtain assays. We present cryo-EM structures of an ATAD2 family ATPase to atomic resolution in three different nucleotide states, revealing unique structural features required for histone loading on DNA, and directly visualize the transitions of Abo1 from an asymmetric spiral (ATP-state) to a symmetric ring (ADP- and apo-states) using high-speed atomic force microscopy (HS-AFM). Furthermore, we find that the acidic pore of ATP-Abo1 binds a peptide substrate which is suggestive of a histone tail. Based on these results, we propose a model whereby Abo1 facilitates H3–H4 loading by utilizing ATP.
Chromatin is a dynamic structure that undergoes significant structural changes during DNA replication, transcription, and repair. This necessitates the regulated assembly and disassembly of nucleosomes by correct deposition of histones or removal of histones from DNA. Nucleosome assembly occurs by a stepwise process where initial binding of H3–H4 histones onto DNA forms a tetrasome intermediate, and subsequently two H2A–H2B dimers are incorporated in a stepwise manner to form the hexasome and nucleosome, respectively. Nucleosome disassembly, on the other hand, is thought to proceed by the reverse pathway of assembly (reviewed in ref. 1).

In order to carefully control nucleosome assembly and disassembly, histone chaperones act as molecular escorts that prevent histone aggregation and undesired nucleic acid interactions. Dysfunction of histone chaperones affects genome stability and gene expression, and can ultimately result in developmental disorders and cancer.2–3

ATAD2 (also termed ANCCA) is a histone chaperone that has been implicated in nucleosome density regulation by histone H3–H4 loading or removal. It is highly overexpressed in various cancers and associated with poor patient prognoses.4–6 Genetic evidence relying on ChIP-seq and RNA-seq experiments suggests that ATAD2 increases chromatin dynamics and gene transcription.7 The budding yeast homolog of ATAD2, Yta7, localizes to highly transcribed regions and decreases nucleosome density, implying that Yta7 evicts H3–H4 to facilitate transcription. In contrast, it was reported that the fission yeast homolog, Ab1, promotes nucleosome occupancy and positioning, potentially by catalyzing assembly of H3–H4 onto DNA. This, thus, the precise biological role of the ATAD2 family is still debatable and remains to be determined.

Although histone chaperones responsible for nucleosome assembly and disassembly usually do not contain ATPase domains, ATAD2 is unique because it is the only known AAA+ ATPase histone chaperone. AAA+ ATPases are a highly conserved family of oligomeric ring-shaped motors that utilize ATP energy to remodel substrates.7,11 The most classical functions associated with AAA+ ATPases are substrate unfolding or disruption of protein–protein interactions—activities that are performed by pulling on substrates through the central pore of the AAA+ ring. ATAD2 has two AAA+ domains in addition to a bromodomain that recognizes histones,12 thus spawning the idea that ATAD2 might perform work on histones analogous to other AAA+ ATPases. Despite such speculation, the biochemical activity and overall structure of any ATAD2 family member have been elusive.

Here, we demonstrate ATP-dependent histone H3–H4 deposition onto DNA by Ab1, the fission yeast ortholog of ATAD2, using an in vitro single-molecule imaging technique, the DNA curtain. We solve the cryo-EM structures of Ab1 in three different nucleotide states (apo, ADP, and ATP), revealing a major reorganization of the AAA+ domains from an open spiral to a closed ring—a structural change that can also be recapitulated in real time by high-speed atomic force microscopy (HS-AFM). Furthermore, we discover a mechanism by which Ab1 accommodates histone substrates, ultimately allowing it to function as a unique energy-dependent histone chaperone.

**Results**

Ab1 is an ATPase that interacts with histone H3–H4. In order to directly determine the activity of an ATAD2 family ATPase in vitro, we recombinantly purified a near full-length version of *Schizosaccharomyces pombe* Ab1 where only the acidic N-terminus was truncated. The construct encompassed the two AAA+ domains, the bromodomain, and a C-terminal domain, all of which are structurally well conserved in the ATAD2 family (Fig. 1a and Supplementary Fig. 1). The profile of the purified

**Fig. 1** Recombinant Ab1 is an ATPase that binds histone H3–H4. a Conserved domain organization of *S. pombe* Ab1 and human ATAD2. We term the region between the AAA2 domain and bromodomain (shown in gray) the “linker arm” based on structural data shown below (Fig. 3b, c). b Gel filtration profile of Ab1 over a Superose6 column under different buffer (2 mM EDTA, 2 mM MgCl2, and 2 mM MgATP) conditions. c Binding of Ab1 to Cy3-labeled H3–H4 measured by fluorescence anisotropy assays. The Kd of Ab1 is 23 ± 13 nM. Error bars represent the standard error of the mean (SEM) for three experiments with different preparations of protein. d Steady-state ATPase rate of Ab1 in the presence of histone substrates. Error bars represent SEM for three experiments with different preparations of protein.
recombinant protein exhibited a homogeneous distribution on a gel filtration column which corresponded to the size of a hexamer, regardless of the presence or absence of nucleotide (Fig. 1b). This was in contrast to other AAA+ ATPases that usually form monomers in the absence of nucleotide.

Consistent with previous genetic studies⁸,⁹, we found that recombinant Abo1 bound specifically to histone H3–H4 with nanomolar affinity (Kd of ~23 ± 13 nM, Fig. 1c) using fluorescence polarization with Cy3-labeled histone H3–H4 (Cy3-H3–H4*). The affinity did not change significantly in the presence of nucleotide, suggesting that the Abo1-histone interaction is not particularly sensitive to ATP. Enzymatically, Abo1 displayed a steady state ATPase rate of 0.83 ± 0.07 ATP/hexamer/s) that was unchanged by the addition of histones (Fig. 1d). These data together show that Abo1 is an ATPase that tightly interacts with histone H3–H4.

ATP hydrolysis-dependent H3–H4 loading onto DNA by Abo1. Although we confirmed that Abo1 is an ATPase interacting with H3–H4, it was unclear whether Abo1 is involved in assembly or disassembly of histones. To directly visualize the process of histone H3–H4 loading or unloading on DNA, we adopted a single-tethered DNA curtain assay, which allows real-time imaging of fluorescently-labeled proteins bound to individual DNA molecules in a microfluidic chamber using total internal reflection fluorescence microscopy (Fig. 2a)⁰,¹⁰. By adding Cy5-labeled H3–H4 to DNA curtains and switching flow
on and off, we were able to image the specific attachment of histones to DNA (Fig. 2b, c). When histone H3–H4 mixed with Abo1 (w/Abo1) and ATP (+ATP) was flowed into a DNA curtain, DNA molecules were decorated with histones as shown by the appearance of Cy5 signal (red), whereas the absence of Abo1 (w/o Abo1) showed nearly no DNA binding (Fig. 2d, e and Supplementary Movies 1–2).

Because Abo1 is an AAA+ ATPase, we next asked whether the H3–H4 loading activity of Abo1 is nucleotide-dependent. While we observed that ~85% of DNA molecules were loaded with H3–H4 in the presence of Abo1 and ATP, less than 10% of DNA molecules were bound to H3–H4 in the absence of ATP, suggesting that ATP is required for Abo1-dependent H3–H4 loading onto DNA (Fig. 2f, g). The addition of ADP, non-hydrolyzable ATP analogs (ATPγS and AMPPPNP), or the use of an Abo1 Walker B mutant (E372Q) also inhibited H3–H4 loading onto DNA, indicating that ATP hydrolysis, rather than ATP binding, is critical for Abo1 to deposit histones onto DNA. These data show that Abo1 directly loads histone H3–H4 onto DNA in the presence of ATP.

We then analyzed the positions of loaded H3–H4 by Abo1 on lambda DNA (λ-DNA) to examine if Abo1 has a preference for specific DNA sequences. The H3–H4 binding distributions showed no preference for binding position, demonstrating that Abo1 deposits H3–H4 on DNA in a sequence-independent manner (Supplementary Fig. 2a) as expected for a general histone chaperone.

In order to probe the characteristics of the histone–DNA complexes assembled by Abo1, we next performed Mnase digestion assays (Supplementary Fig. 2b), to see if histones loaded by Abo1 could confer Mnase protection. CAF-1, a well-characterized histone chaperone that promotes tetrasome assembly15, is characterized histone chaperone that is known to assemble tetrasomes assembled by Abo1, we next performed Mnase digestion assays (Supplementary Fig. 2b), to see if histones bound by Abo1 could confer Mnase protection. CAF-1, a well-characterized histone chaperone that promotes tetrasome assembly15, is characterized histone chaperone that promotes tetrasome assembly15.

Abo1, in contrast, did not protect 70–80 bp DNA fragments but showed a distinct Mnase digestion pattern, where long DNA fragments (~150 bp) and short DNA fragments (<50 bp) were protected. We also observed protection of the short DNA fragments with Abo1 alone, implying that Abo1 binds to DNA even in the absence of histones, and that the long protected fragments result from DNA binding of Abo1–H3–H4 complexes. Taken together, these results support the idea that Abo1 loads histones onto DNA in a distinct manner from conventional tetrasomes.

Besides loading histones onto DNA, we also asked whether Abo1 can unload histones from DNA curtains, because studies of the human and budding yeast counterparts of Abo1 show that nucleosome density decreases in the absence of these proteins. We assembled H3–H4 onto DNA with yeast CAF-1 (yCAF-1) and examined if the level of assembled H3–H4 decreased when Abo1 was added. When Abo1 is added to DNA-histone curtains, we found no significant reduction in the fluorescence intensity of histones nor the fraction of histone-bound DNA (Supplementary Fig. 3 and Supplementary Movies 3–4). Thus, Abo1 catalyzes histone H3–H4 deposition onto DNA but does not directly promote removal of H3–H4 from DNA, which is consistent with previous in vivo studies showing that Abo1 is involved in chromatin assembly and organization9.

### Cryo-EM structure of Abo1 in the ATP state

To gain further insight into the molecular mechanism by which Abo1 couples ATP hydrolysis to histone loading, we determined the cryo-EM structures of Abo1 in three different nucleotide states (ATP-bound and ADP-bound states, and the apo state). We first solved the cryo-EM structure of Abo1 in an ATP-bound state to 3.5 Å resolution using an ATP-hydrolysis deficient Abo1 Walker B mutant (E372Q) (Fig. 3, Supplementary Fig. 4 and Supplementary Table 1). The high quality of the cryo-EM map enabled us to resolve most amino acid side chains (Supplementary Fig. 5), and build a de novo model of Abo1 without applying knowledge from previously solved AAA+ structures.

The atomic resolution model revealed two layers of AAA+ domains arranged in a hexameric spiral (Fig. 3a), with the AAA1 domains forming the top layer and the AAA2 domains the bottom layer. The C-terminal domain was positioned underneath the AAA2 domain. We assigned the region of weak density lying above the plane of AAA1 to the bromodomain, because this is the only globular domain unaccounted for in our structural model.

Densities for six bound nucleotides were found at the interface of AAA1 protomers (Supplementary Fig. 6). We assigned five of the nucleotide densities as ATP, but the identity of one nucleotide (in subunit A) could not be determined with certainty although we modeled this nucleotide as ADP based on superposition with the ADP state (see below). No nucleotide density was found at the interface of the AAA2 domains, consistent with the fact that AAA2 has no consensus Walker A or B motifs and is presumably catalytically inactive.

The structural fold of the Abo1 AAA+ domain is overall similar to those of other AAA+ proteins in the classical clade10, where the nucleotide binding α/β domain (NBD) and the helix bundle domain (HBD) are well conserved. However, superposing Abo1 with other AAA+ ATPases highlights non-canonical features that distinguish it from other AAA+ ATPases (Fig. 3c).

First, Abo1 subunits have a molecular clamp where the “knob” of one subunit locks into a “hole” of the neighboring subunit by an electrostatic interaction (Fig. 3b–d, Supplementary Fig. 7). The “knob” is formed by a helix-turn-helix insert between the first helix (α0) and first sheet (β1) of AAA2 NBD (Supplementary Fig. 8), a feature that is not observed in other AAA+ ATPases. The “hole” is formed by the AAA1 HBD, AAA2 HBD, and a linker that proceeds the AAA2 HBD (Fig. 3c). This unique addition, which we name the “linker arm” (Figs. 1a and 3b, c), stabilizes the hexameric conformation and explains how Abo1, in contrast to other AAA+ ATPases, can exist as a stable hexamer even in the absence of nucleotide (Fig. 1b).

Second, the AAA2 HBD consists of two distinct parts that originate from distant parts of the primary structure. Helix 5 (α5) of AAA2 HBD extends directly out of the AAA2 NBD, but helices 6–8 (α6–α8) of the AAA2 HBD originate from the Abo1 C-terminal domain (Fig. 3b, e). Thus, what was originally identified as the C-terminal domain12 is in fact a part of AAA2 HBD.

Finally, Abo1 has two linker regions of significant length that join the AAA+ domains and substrate binding bromodomains in an unusual arrangement (Fig. 3b). A ~40aa linker arm (aa 755–775) protrudes out from the first helix of AAA2 HBD on the bottom of the AAA+ ring and extends toward the substrate binding bromodomain on top of the AAA+ ring. Another linker that we observe weak density for, presumably extends out of the bromodomains on the top of the molecule, and inserts back into the second helix of AAA2 HBD on the bottom of the AAA+ ring. These two linkers form structures that span the side of the AAA+ ring in opposite directions, and connect the bromodomains and AAA2 domains.

### Nucleotide-dependent structural changes of Abo1

To dissect nucleotide-dependent structural changes of Abo1, we next determined the cryo-EM structure of Abo1 in the ADP state to 4.4 Å resolution. In addition, we determined two cryo-EM
structures in apo-states. One structure was determined to 4.3 Å resolution, which is similar in resolution to ADP-Abo1. The other structure that showed clear features of the bromodomain was determined to 6.9 Å resolution from data collected using a Volta phase plate. Therefore, we used the 4.3 Å resolution structure of apo-Abo1 for comparing with ATP- or ADP- bound states, and referred to the lower resolution structure of apo-Abo1 for describing the bromodomain (Fig. 4a, Supplementary Figs. 9, 10 and 11 and Supplementary Table 1). We then performed flexible fitting of the ATP-model into the ADP- and apo- cryo-EM maps with MDFF16 (Fig. 4b and Supplementary Fig. 13). Comparison of the ATP-Abo1 Walker B mutant structure with the ADP- and apo-states revealed striking differences in the subunit arrangement, heights and individual subunit conformation (Fig. 4b),

Fig. 3 Cryo-EM structure of Abo1-Walker B mutant in the ATP state shows unique AAA+ structural organization. a An atomic model (left) and cryo-EM map (right) of ATP-Abo1 Walker B mutant viewed from “bottom” (AAA2 side, top panel) and “side” of the AAA+ ring (bottom panel). The AAA1 domain is colored in light green, the AAA2 domain in teal, and the C-terminal domain in magenta. From the side view, the bromodomain, AAA1, AAA2, and the C-terminal form four tiers. The scale bars indicate 2 nm (20 Å). b The structure of a single Abo1 monomer within the Abo1 hexamer. Helices of AAA1 and AAA2 NBD and HBD are labeled according to AAA+ structural convention. Approximate bromodomain position based on electron density maps is depicted as a cartoon oval. The connectivity of the AAA+ domain, bromodomain, and C-terminal domain based on the structure and cryo-EM map is indicated as dotted lines. c Superposition of the Ca’s of the Abo1 backbone (dark blue) with the AAA+ ATPases p97 (light green, PDB ID: 5FTM) and NSF (pink, PDB ID: 3J94), aligned with respect to AAA1. The unique inserts of Abo1—the AAA2 knob and the linker arm proceeding AAA2 α5—are highlighted by shaded ovals. The AAA2 domains do not align well due to the variable angle between AAA1 and AAA2. d The “knob and hole” packing of Abo1 subunits where the knob of one subunit inserts into the hole of the adjacent subunit (black arrowheads and dotted circles). Abo1 subunits are colored by chain. e The “split” AAA2 helical bundle domain (HBD) of Abo1, where α5 of AAA2 (teal, aa 734–750) interacts with three helices (α6–α8) of the C-terminal domain (magenta, aa1129–1185). AAA2 HBD is superposed onto its closest structural relative, 26 S proteasome AAA-ATPase subunit 6B (tan, PDB ID: 5ln3), showing that the three-dimensional structure of Abo1 HBD is conserved with other AAA+ HBD’s despite unique helix connectivity.
while the structures of the ADP- and apo-states were similar to each other.

In the ADP state, the subunits assumed similar heights forming a symmetric planar ring, whereas in the ATP-state, the subunits were staggered in height and shifted towards the center forming an asymmetric spiral with a smaller pore. The most prominent structural change could be observed at the interface of subunits A and F where in the ADP state (Fig. 5a, b), these two subunits maintained similar heights with close inter-subunit packing, while in the ATP state, the subunits were staggered in height and separated by ~40 Å.

To further dissect the details of this conformational change we aligned the individual subunits of Abo1 in the ADP- and ATP-states. Individual subunits in the ADP state did not show any significant differences in their structures, supporting the idea that ADP-Abo1 is largely symmetric. However, individual subunits in the ATP state displayed a marked difference where the AAA1 domain and the linker arm progressively shift away from the AAA2 domain as the subunits rise in height along the spiral axis (Fig. 5c). When comparing the bottom-most subunit of the spiral (subunit A) with the top-most subunit (subunit F), the distance between the AAA1 and AAA2 NBD increases by 4.9 angstroms, and the axis between the AAA1 and AAA2 NBD tilts 10 degrees with respect to the AAA2 HBD. This implies that the junction between AAA1 and AAA2 is flexible and contributes to the variability of AAA1-AAA2 angles. In addition, linker arm flexibility can change the shape of the “hole” in the interlocking “knob-and-hole” structures. Notably, the density for the linker arm in the bottom-most subunit (subunit A) and the density for the “knob” in the top-most subunit (subunit F) were not discernible, as the subunits do not interlock at this interface.

When comparing the two extreme conformations of subunits (subunit A and subunit F) in the ATP-bound structure with the conformation of the subunit in the ADP-bound structure, we found that the bottom-most subunit in the ATP-bound structure (subunit A) superimposed well with the subunit in the ADP-bound structure (Fig. 5d), while the top-most subunit (subunit F) diverged significantly from the ADP-conformation. All other subunits assumed positions that clustered in between these two extremes. Therefore, the variability observed in the individual ATP subunits also provides the basis for the conformational change between the ATP and ADP states. Based on this information, the bottom-most subunit is likely in a post-hydrolysis ADP state, which is also in agreement with other asymmetric spiral AAA+ ATPases.

Lastly, but more interestingly, in contrast to the ATP-Abo1 structure, the bromodomain density was stronger and more symmetric in the ADP- and apo-structures (Fig. 4a). Especially in the low resolution cryo-EM structure of the apo-state, six lobes of density that match the expected dimensions of a bromodomain were arranged in a hexameric ring around a central lobe of density. On the outer face of the bromodomain ring, characteristic helical densities that run diagonal to the central ring axis could be observed (Supplementary Fig. 12). However, due to the low resolution of this region, we were unable to fit bromodomain homology models with confidence, nor assign secondary structures. In addition, we also observed an extra density at the center of the bromodomains, although it is unclear whether this is part of Abo1 or a bound substrate. Collectively, the cryo-EM structures in three different nucleotide states reveal substantial structural change in Abo1 upon ATP hydrolysis which likely plays a critical role in loading histones on DNA, and also suggest that structural changes in the AAA+ ring may be coupled to changes in the organization of the bromodomains.

**Fig. 4 Comparison of Abo1 cryo-EM structures in the ATP and ADP states reveal a hexameric spiral-to-ring transition.** a Cryo-EM maps of Abo1 in the ATP, ADP, and apo states showing the “top” (AAA1), “side”, and “bottom” (AAA2) face of the AAA+ hexamer. Resolution of ATP, ADP, and apo structures are at 3.5 Å, 4.4 Å, and 6.9 Å respectively. Electron density above the AAA+ ring is assigned as the bromodomain. The top view is clipped underneath the bromodomain to show the upper surface of the AAA1 domains. A top view of the apo-Abo1 bromodomain is shown in Supplementary Fig. 12. b Structures of Abo1 in the ATP state, and flexible-fitted Abo1 models in the ADP and apo state. The Abo1 hexamer is colored according to subunit (chains A–F).
determined by AFM matched well with dimensions from our cryo-EM structures, and likely correspond to the C-terminal and AAA2 side of Abo1, as they show hexameric lobes and a central pore that recapitulate pseudo-AFM images simulated using a hard sphere model of the bottom (AAA2) surface of Abo1 but not the top (AAA1) surface (Fig. 6c).

Real-time imaging of wild-type Abo1 in the presence of 2 mM ATP revealed striking symmetry breaking events, where individual blades of the hexameric ring seemed to disappear due to a decrease in the height of a subunit (Fig. 6d and Supplementary Movies 5 and 6). In most asymmetric states, only one blade disappeared from the field of view, but there were also rare transient cases where two blades of the ring disappeared simultaneously (Supplementary Fig. 14). These HS-AFM results were highly consistent with our cryo-EM structures that show a closed symmetric ring for apo- and ADP states and an open spiral in the ATP-state. Dwell time analysis of ring opening and closing measured a rate of $1.5 \text{s}^{-1}$ for opening and $0.99 \text{s}^{-1}$ for closing, rates that approximately agree with bulk ATPase rates (Supplementary Fig. 15). Interestingly, the Abo1 Walker B mutant displayed only single symmetry breaking events in the presence of ATP, such that it is "stuck" in the asymmetric spiral conformation (Supplementary Fig. 16; Supplementary Movie 7).

In order to determine how Abo1 utilizes ATP, we tracked the position of ring openings, which indicate which subunit is activated and hydrolyzes ATP. In tracking multiple molecules, we...
found that the ring opening takes place randomly without an ordered sequence (Fig. 6e; Supplementary Fig. 17), indicating that Abo1 subunits are randomly activated. This suggests that at least under basal conditions without added substrate, Abo1 subunits hydrolyze ATP stochastically as proposed for the AAA+ protease ClpX.\(^{18}\)

**Abo1 binds histones with the AAA+ pore and the bromodomain.** Unexpectedly, after building an atomic model into the cryo-EM map of the ATP-Abo1, we discovered a lobe of extra density in the central pore that could not be accounted for by any part of Abo1 (Fig. 7a). Comparison with the central pore of the ADP- or apo-states showed that this density was unique to the ATP-Abo1 state. We modeled this density as a 14-alanine chain, and examination of interactions between the polyalanine chain and Abo1 revealed that the W345 sidechains of Abo1 form a winding tryptophan staircase around the peptide (Fig. 7b), where the middle subunits (B–E) form direct contacts. The bottom-most tryptophan (subunit A) is completely dislodged from the peptide, while the top-most tryptophan (subunit F) side chain faces away. This type of structure was similar to that seen with other AAA+...
ATPases binding substrates such as the tyrosine staircase seen in Hsp104 binding to casein19 or the tryptophan staircase in Vps4 binding to ESCRT-III20,21. Alignment of Abo1 AAA1 with other AAA+ ATPases shows that although the amino acid type is different in different AAA+ ATPases, the position of the key substrate binding residue side chain in pore loop1 is well conserved (Fig. 7c). Based on these observations and similar reports of co-purifying substrates in other AAA+ ATPases22, we attributed the extra density to the binding of an unknown endogenous substrate(s) such as histones from insect cells that had been inadvertently co-purified with Abo1.

Upon closer examination of Abo1 pore loops, we found two acidic amino acid side chains—E385 of AAA1 pore loop2 and D642 of AAA2 pore loop1—facing the pore interior, thus creating a negatively charged pore interior contributed by acidic pore loop residues.
a negatively charged surface (Fig. 7d). Considering that Ab1 is a histone chaperone, we suspected that some of the extra density in the central pore might correspond to histones or other basic proteins. We were unable to identify any significant hits by mass spectrometry of the cryo-EM Ab1 sample, but when we performed crosslinking-mass spectrometry with Ab1 and H3–H4 (Supplementary Fig. 18), we found many intra- and intermolecular crosslinks (Supplementary Tables 2 and 3). Due to the complexity of the interpretation of intra-molecular crosslinks with the Ab1 homohexamer, we focused on analyzing the intermolecular crosslinks between histones and Ab1, most of which mapped to the Ab1 bromodomain (Fig. 7e and Supplementary Data 1). Interestingly, the highest confidence intermolecular crosslink was between the Ab1 pore loop and the histone H3 N-terminal tail implying that Ab1 might bind histones by the central pore as well as the bromodomain.

To test the potential significance of Ab1 pore—histone tail interactions, we investigated the effect of pore loop mutations and histone tail truncations on the histone loading activity of Ab1. Two Ab1 mutants of conserved pore loop residues (W345A and E385A) displayed very low histone deposition activity in our DNA curtain assay (Fig. 7f, g) despite similar ATPase rates and no significant difference in histone binding compared to wild type (Supplementary Fig. 19). Removing histone N-terminal tails of histone H3–H4 by trypsinization (Fig. 7f, g, and Supplementary Fig. 20) also abolished histone loading on DNA curtains in a similar manner. Therefore, these data suggest that the pore loop residues and histone N-terminal tails are essential for chaperone activity of Ab1.

Because our cryo-EM structure of Ab1 in the apo state shows that the bromodomains form a hexameric ring above the AAA+ ring, we also investigated the contribution of the bromodomains to Ab1 histone loading activity. By mutating E900, a residue that is predicted to be required for histone binding, we found that the E900A bromodomain mutant failed to load histones in the DNA curtain assay (Fig. 7f, g). This implies that both the bromodomains and the pore function in loading histones on DNA by Ab1.

Discussion

Our studies establish Ab1 as an ATP hydrolysis-dependent histone chaperone, and support in vivo data from S. pombe where Ab1 promotes nucleosome occupancy9. Interestingly, previous studies in vivo studies with human (ATAD2) and yeast orthologs (Yta7) proposed that AAA+ histone chaperones evict nucleosomes rather than promote assembly7,8,23. Although here we show that Ab1 directly mediates H3–H4 loading onto DNA, it remains to be determined whether there is a true difference between species, or whether Ab1 can function both to promote histone assembly and disassembly depending on the biological context as has been proposed for other ATP-independent histone chaperones such as FACT24–26.

Our DNA curtain analyses clearly demonstrate that Ab1 facilitates H3–H4 loading onto DNA in an ATP-dependent manner. MNase treatment of Ab1 assembled H3–H4–DNA complexes show a distinct pattern of protection when compared to CAF-1 assembled H3–H4–DNA complexes. These data suggest that Ab1 loads histones to DNA in a conformation that may be distinct from tetrasomes assembled by CAF-1. It is possible that Ab1 simply loads H3–H4 onto DNA, and requires other factors to assemble proper tetrasomes, or that Ab1-assembled H3–H4 complexes represent an alternate conformation of histone–DNA complexes.

The cryo-EM structures presented in this work reveal that Ab1 assumes a distinct structure that consists of two main parts—a AAA+ ring base and a bromodomain top—that cooperate to bind histones. The bromodomains mimic the 6-fold symmetry of the AAA+ base and assemble into a hexameric ring, thus creating a unique pocket where histones could bind. This arrangement has not been observed with any other bromodomain protein, and is in stark contrast to other histone chaperones which commonly utilize a four-stranded β-structure28 to bind histones in an ATP-independent manner. Based on our structure and the tighter H3–H4 binding affinity of Ab1 compared to other conventional bromodomains, we speculate that H3–H4 forms a broad binding interface with Ab1 consisting of the inner lining of the bromodomains and the AAA+ ring pore. This is consistent with studies in budding yeast Yta7 (ref. 27), where the authors found that Yta7 binds histones by regions other than the bromodomains.

Not only is Ab1 distinct as a histone chaperone, but Ab1 is also unique compared to other canonical AAA+ protein structures. While most other AAA+ proteins show dynamic assembly and disassembly of hexameric rings depending on the presence of nucleotides, the Ab1 hexamer is stabilized by structural inserts that mediate the knob-and-hole packing of individual subunits. Furthermore, AAA2 HBD has a bromodomain inserted with long linkers in between helix 5 and 6 of the HBD, highlighting the versatility of the AAA+ fold.

Analysis of nucleotide-dependent structural changes of Ab1 reveals a striking conformational shift of Ab1 upon ATP hydrolysis that is required for histone loading onto DNA. The spiral-to-ring reorganization is similar to that seen in recent structures of other AAA+ ATPases such as Hsp104 NSF19,28 and the proteasome29, although the exact nucleotide state of Ab1 subunits differs. We speculate that such nucleotide-dependent changes in AAA+ ring structure regulate the structure of the bromodomain ring, as the bromodomain density in the apo and ADP states is more distinct compared to the ATP state.

By real-time analysis of Ab1 conformational change, we have directly shown that ATP hydrolysis in Ab1 occurs only in one or two subunits at a time suggesting that ATP hydrolysis in one subunit suppresses ATP hydrolysis in other subunits. We have also shown that in the absence of substrate, there is no ordered sequence of subunit activation, and that the position of symmetry breaking is random. Future studies should elucidate whether this mode of stochastic ATP utilization is maintained even with bound H3–H4 substrate, or whether Ab1 transitions to a sequential mode of ATP hydrolysis analogous to mechanisms proposed for Yme1p17 and Hsp104 (ref. 28).

Based on our cryo-EM structures, our current model for Ab1-dependent histone loading on DNA can be summarized as follows. In the ATP-state, Ab1 assumes a closely packed spiral that binds the histone H3 N-terminal through its pore loops and the histone body through its bromodomain. The central pore of Ab1 is ~13 Å in diameter and can accommodate binding of only one histone H3–H4 dimer. When ATP hydrolysis occurs, Ab1 transitions to a planar ring with a larger pore, potentially allowing for remodeling and/or release of histone H3–H4. In this process, we speculate that the energy of ATP hydrolysis might be utilized for exposing the correct histone surfaces and/or to remodel histones to a favorable conformation for DNA deposition. Regardless of the exact role, ATP hydrolysis is essential for Ab1 to load a single H3–H4 dimer onto DNA to initiate nucleosome loading. Alternatively, it is also possible that two Ab1 molecules simultaneously deliver two H3–H4 dimers to DNA in resemblance to other H3–H4 assembly chaperones such as CAF-1 and Rtt10630,31.

Despite sharing a common structure with other AAA+ ATPases that function as protein disassembly machines, Ab1
performs a different role in facilitating the assembly of higher order structures. This seems to be more akin to the AAA+/ATPase Rvβ1/Rvβ2-assisted assembly of INO80 (ref. 22) or the ClpX induced remodeling and activation of ALA synthase23. Further structural studies on Abo1-histone complexes should shed light on how the Abo1 AAA+ ATPase uses energy to guide the initial process of nucleosome assembly. In summary, our work demonstrates the previously unobserved process of ATP-dependent histone deposition and alludes to the possibility that there might be a nucleosome assembly step that requires ATP energy.

Methods

Cloning and protein expression of Abo1. The gene encoding Abo1 aa23–1190 was synthesized (GenSCRIPT) and cloned into a FastBac HTB vector with an N-terminal 6×His tag and TEV protease cleavage site. Point mutants of Abo1 were created by inverse PCR with KOD plus polymerase (Toyobo), followed by digestion with DpnI, phosphorylation with T4 phosphonucleotidase kinase, and ligation with T4 ligase. Bacmidas were prepared from transformed DH10Bac competent cells and transfected into SF9 cells to produce recombinant Baculovirus (Nikon). Cy5 was collected by a 60× water immersion objective (CFI Plan Apo VC 60XWI, Nikon).

Fluorescent histone preparation. Wild-type Xenopus histones were purified by inclusion body preparation as described previously24. Lyophilized H4T71C was removed by dialysis and refolded with wild type H3 in refolding buffer (20 mM Tris-HCl pH 8.0, 300 mM NaCl, 5 % glycerol (Roche), and lyzed by 4 cycles of freezing and thawing. Cell lysates were subsequently cleared by centrifugation at 39,000 g for 1.5 h, and bound to Ni-NTA resin (Qiagen) pre-equilibrated with lysis buffer for 2 h. Ni-NTA resin was washed with wash buffer (lysis buffer supplemented with 20 mM imidazole) and elution buffer (lysis buffer supplemented with 100 mM imidazole). Eluted fractions were pooled and cut with TEV protease overnight in dialysis buffer (50 mM Tris- HCl pH 8.0, 250 mM NaCl, 5 % glycerol, 1 mM DTT). Contaminants and uncleaved His-tagged protein were removed by cycling over Ni-NTA resin 5 times, and further purified by running a 50 mM to 1000 mM NaCl gradient over a HiTrap Q 5 ml column (GE Healthcare). Typical yields were -50 μg of protein per liter culture of insect cells.

Fluorescent histone anisotropy. The fluorescence anisotropy of Cy3-labeled H3–H4 was measured on a SpectraMax M5 Multi-Mode Plate Reader (Molecular Devices) using a fluorescence anisotropy assay as described previously. Abo1 was added to 20 nM Cy5-labeled histone H3–H4. All measurements were performed at room temperature.

ATPase assays. The EnzChek Phosphate assay kit (Thermo Fisher Scientific) was used to measure steady state ATPase rates of Abo1. Abo1 was incubated in assay buffer (50 mM Tris pH 8.0, 2 mM MgCl2, 100 mM NaCl, 1 mM DTT) with or without histone substrates, and 2 mM ATP was added to initiate the reaction at 30°C. Absorbance at 360 nm was monitored on a Spark multimode microplate reader (Tecan) 5 s after initiating the reaction, and with 10 s intervals thereafter.

Single-molecule DNA curtain assays. DNA curtain assays were performed as previously described19,25. Briefly, flowcells were prepared by sticking a coverslip onto a fused-silica slide with nanofabricated chromium barriers. A biotinylated lipase blader was deposited on the surface by injection of liposomes containing DOPC (1,2-dioleoyl-sn-glycerol-phosphocholine), 0.5% biotinylated-DOPE (1,2-dipalmitoyl-sn-glycerol-3-phosphoethanolamine-N-(cap-biotinyli), and 8% MPEG 2000-DOPE (1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine-N-[methoxy[poly- ethylene glycol]–2000]) (Avanti Polar Lipids). The surface was further passivated with 0.2 mg/ml BSA. Biotinylated X-DNA was anchored on biotinylated lipid via streptavidin. The flowcell was connected to a syringe pump fluidic system and placed on a customized prism-type total internal reflection fluorescence (TIRF) microscope that was built on an inverted fluorescence microscope (Eclipse Ti-2, Nikon).

Solid state lasers 488 nm and 637 nm (200 mW, OBIS, Coherent Laser) were used to excite YOYO-1 and Cy5, respectively. The fluorescence from YOYO-1 or Cy5 was collected by a 60x water immersion objective (CFI Plan Apo VC 60XWI, Nikon) imaged on each of two EMCCD cameras (iXON 897, Andor Technology) after passing the optical filter blocking excitation laser. 

For histone assembly assays, lambda phage DNA was first confirmed by the staining with the intercalating agent YOYO-1. To exclude the possibility that YOYO-1 might interfere with histone binding, YOYO-1 was washed out with assay buffer supplemented with 20 mM MgCl2 and 200 mM NaCl. 5 nM Abo1 and 12.5 nM Cy5–H3–H4 were pre-incubated in buffer supplemented with 1 mM nucleotide cofactor on ice for 15 min, and then injected into the flowcell. Upon the arrival of the maximum amount of proteins to DNA curtains, flow was stopped, and the proteins were incubated with DNA curtains for 15 min. The binding of H3–H4 was achieved by resuming flow. To test Abo1 disassembly activity, we first loaded Cy5–H3–H4 onto DNA using purified yeast CAF-1 (yCAF-1). 5 nM of yCAF-1 was preincubated with 12.5 nM of H3–H4 for 15 min on ice in assay buffer. CAF-1–H3–H4 was injected into the DNA curtain flow cell, incubated for 15 min at room temperature, and washed out with assay buffer for 5 min. Subsequently, 5 nM of Abo1 was injected into the H3–H4-loaded DNA, and movies were taken after 15 min. During all injection and incubation steps, lasers were turned off to prevent Cy5 photobleaching. All DNA curtain movies taken by NIS Elements software were converted to 8-bit TIFF stacks and analyzed in Image J. Kymographs for individual DNA molecules were created, and the fluorescence intensity profile at the tenth frame in each kymograph was obtained. The intensity profile was fitted by multiple Gaussian functions. We analyzed only fluorescence peaks that disappeared when flow was turned off. The peak positions of Gaussians were collected to obtain the binding position distribution of H3–H4.

MNase digestion assays. For MNase digestion assays, H3–H4 histones were assembled on 268 bp Widom 601 sequence DNA. 200 nM DNA was mixed with 500 nM H3–H4 dimers and 4 μM Cy3-Caf1 or 4 μM Abo1 and incubated at 23 °C for 1 h. MNase was treated at a concentration of 5 U/μl for 30–90 min in MNase reaction buffer (50 mM Tris-HCl pH 7.9 and 5 mM CaCl2) at 37°C, and quenched by addition of 0.3 mg/ml protease K and 20 mM EDTA and further incubation for 30 min. Digestion products were extracted with phenol-chloroform, precipitated by ethanol, separated on an 8% non-denaturing gel in 1x TBE buffer, and stained with EtBr.

Cryo-EM grid preparation. Prior to sample vitrification, 1.1 mg/ml thawed Abo1 protein was exchanged into a buffer containing 20 mM HEPES pH7.5, 250 mM NaCl, 1 mM DTT, 0.025 % octyl β-D-glucoside, and either 2 mM MgADP or 2 mM MgATP. 3 μl of Abo1 was loaded onto Quantifoil R1.2/1.3 grids (Quantifoil Micro Tools GmbH) that were glow discharged with 15 mA for 1 min. Grids were blotted for 2–3 s and plunged frozen in liquid ethane cooled by liquid nitrogen using a Vitrobot (Thermo Fisher Scientific).

Cryo-EM data collection. Data were collected at three locations: Korea Basic Science Institute (Ochang, Korea) using a Titan Krios 300 keV with a Falcon III direct detector for Abo1-ATP and ADP-Abo1, SciLifeLabs (Stockholm, Sweden) using a Titan Krios 300 keV with a Gatan K2 Summit detector for apo-Abo1, and eBIC at Diamond Light Source (Didcot, UK) using a Titan Krios 300 keV with a Volta phase plate and Gatan K2 Summit detector for apo-Abo1 structure #2. Details of micrographs, exposure, dose, and pixel size are summarized in Supplementary Table 1.

EM data processing. Image processing for ATP-Abo1, ADP-Abo1, and apo-Abo1 was performed using cisTEM36. Details of the number of selected particles, 2D class averages, initial models, model refinement are summarized in Supplementary Table 1, and the data processing schemes are shown in Supplementary Figs. 4, 9, 10, and 11. All images were motion-corrected with MotionCor2 (ref. 27) and CTF refinement performed in cisTEM. Particle selections were performed non-template based picking in cisTEM. Several rounds of reference-free 2D classification were performed to remove non-particles and poorly aligning particles, until strong secondary structure features of AAA+ domains were visible in the 2D classes. This subset was used to generate reference-free ab initio models, with no applied symmetry (C1), resulting in an initial 3D model. For ATP-Abo1, 3D classification was performed with 4 classes, using the initial 3D model. The highest resolution class was the most highly populated with 122,644 particles. Using this class, we performed another round of 3D classification, selecting the highest resolution class, and an additional round of 2D classification to select the best aligned classes. Homogeneous re-classification was performed using 4 classes, and a final model with a resolution of 3.5 Å (ATP-state). Side chains were visible for the AAA+ domains, but only very weak density was visible for the bromodomains and was of poor quality as the local resolution estimates showed in ResMap38. For ADP-Abo1 and apo-Abo1, initial processing steps up to 3D classification with 4 classes was the same as for ATP-Abo1. However, for these states, further 3D and/or 2D classification, did not increase the resolution of the structures. Thus, only one round of 3D classification followed by final homogeneous refinement and...
sharpening was performed to obtain final models with resolutions of 4.4 Å (ADP state), and 4.3 Å (apo-state) as determined by the gold standard FSC at 0.143 criterion.

Image processing for apo-Abo1 structure #2, which has overall lower resolution than apo-Abo1 structure #1 but improved resolution for the bromodomains, was performed using RELION3.019, using a volta-phase plate collected dataset to improve contrast and particle picking. Particle selection was performed using Laplacian-of-Gaussian filtering autopicking. Reference-free 2D classification, initial model generation, and 3D classification was performed as with other datasets, but selection was based more on strong bromodomain density, rather than the overall resolution that includes the AAA+ domain. 3D classification to 4 classes revealed a class with better bromodomain density than other classes, and a subsequent homogeneous 3D refinement and polishing resulted in a final model with a resolution of 6.9 Å. Attempts to further improve the resolution of this model by the application of C6 symmetry, multi-body refinement, and masking were unsuccessful.

Atomic model building and refinement. The atomic model of ATP-Abo1 Walker B mutant was built in Coot de novo without the use of any homology templates. The model for chain E, the chain which had the clearest electron density, was built first. Subsequently this model was used as a template to build and refine model other chains. The final model was refined and validated by using cryo-EM validation tools in Phenix.14

Molecular dynamics flexible fitting. Initial atomic models of apo- and ADP-Abo1 were built by taking copies of the chain “E” in the de novo model of ATP-Abo1 Walker B mutant, which has the most complete build achieved at highest resolution among all chains of A-F. The initial models were docked into the apo- and ADP- cryo-EM density maps using the Fit in Map module of UCSF Chimera40, by relaxing each of the six chains independently. The fits of the atomic models into the low resolution respectively. The atomic models of the ADP-state have been docked into the Protein Data Bank under accession number (EMD-9872 and 9870 for ATP and ADP respectively, and 9871 and 0800 for apo-Abo1 high resolution and apo-Abo1 low resolution respectively). The atomic models of the ATP-state has been deposited to the Protein Data Bank under accession number (PDB ID: 6JQ0, 6PQ0, 6GPU for ATP, ADP, and apo-Abo1, respectively).

Data and materials availability

EM maps are deposited to the Electron Microscopy Data Bank under accession number (EMD-9872 and 9870 for ATP and ADP respectively, and 9871 and 0800 for apo-Abo1 high resolution and apo-Abo1 low resolution respectively). The atomic models of the ATP-state has been deposited to the Protein Data Bank under accession number (PDB ID: 6JQ0, 6PQ0, 6GPU for ATP, ADP, and apo-Abo1, respectively).

Received: 18 April 2019; Accepted: 20 November 2019; Published online: 17 December 2019

References

1. Das, C., Tyler, J. K. & Churchill, M. E. The histone shuffle: histone chaperones in an energetic dance. Trends Biochem. Sci. 35, 476–489 (2010).

2. Gurard-Levin, Z. A., Quivy, J. P. & Almouzni, G. Histone chaperones: assisting histone traffic and nucleosome dynamics. Annu. Rev. Biochem. 83, 487–517 (2014).

3. Burgess, R. J. & Zhang, Z. Histone chaperones in nucleosome assembly and human disease. Nat. Struct. Mol. Biol. 20, 14–22 (2013).

4. Caron, C. et al. Functional characterization of ATAD2 as a new cancer/testis factor and a predictor of poor prognosis in breast and lung cancers. Oncogene 29, 5171–5181 (2010).

5. Kalashnikova, E. V. et al. ANCCA/ATAD2 overexpression identifies breast cancer patients with poor prognosis, acting to drive proliferation and survival of triple-negative cells through control of B-Myb and EZH2. Cancer Res. 70, 9402–9412 (2010).

6. Zou, J. X., Revvenko, A. S., Li, L. B., Gerno, A. T. & Chen, H. W. ANCCA, an estrogen-regulated AAA-ATPase coactivator for ERalpha, is required for coregulator occupancy and chromatin modification. Proc. Natl. Acad. Sci. USA 104, 18067–18072 (2007).

7. Morozumi, Y. et al. ATAD2 is a generalist facilitator of chromatin dynamics in embryonic stem cells. J. Mol. Cell Biol. 8, 349–362 (2016).

8. Lombardi, L. M., Ellahia, A. & Rine, J. Direct regulation of nucleosome density by the conserved AAA-ATPase Yta7. Proc. Natl. Acad. Sci. USA 108, E1302–E1311 (2011).

9. Gal, C. et al. Abol, a conserved bromodomain AAA-ATPase, maintains global nucleosome occupancy and organisation. EMBO Rep. 17, 79–93 (2016).

10. Erzberger, J. P. & Berger, J. M. Evolutionary relationships and structural mechanisms of AAA+ proteins. Annu. Rev. Biophys. Biomol. Struct. 35, 93–114 (2006).

11. Vale, R. D. AAA proteins. Lords of the ring. J. Cell Biol. 150, F13–F19 (2000).

12. Cattaneo, M. et al. Lessons from yeast on emerging roles of the ATAD2 protein family in gene regulation and genome organization. Mol. Cells 37, 851–856 (2014).

13. Greene, E. C., Wind, S., Fazio, T., Gorman, J. & Visnapuu, M. L. DNA curtains for high-throughput single-molecule optical imaging. Methods Enzymol. 472, 293–315 (2010).

14. Fazio, T., Visnapuu, M. L., Wind, S. & Greene, E. C. DNA curtains and nanoscale curtain rods: high-throughput tools for single molecule imaging. Langmuir 24, 10524–10531 (2008).

15. Sauer, P. V. et al. Mechanistic insights into histone deposition and nucleosome assembly by the chromatin assembly factor-1. Nucleic Acids Res. 46, 9907–9917 (2018).
16. Trabuco, L. G., Villa, E., Mitra, K., Frank, J. & Schulten, K. Flexible fitting of atomic structures into electron microscopy maps using molecular dynamics. Structure 16, 673–683 (2008).

17. Puchades, C. et al. Structure of the mitochondrial inner membrane AAA+ protease YME1 gives insight into substrate processing. Science 358, https://doi.org/10.1126/science.aao4617 (2017).

18. Glynis, S. E., Martin, A., Nager, A. R., Baker, T. A. & Sauer, R. T. Structures of asymmetric Chaperones reveal nucleotide-dependent motions in the AAA+ protein-unfolding machine. Cell 139, 744–756 (2009).

19. Yokom, A. L. et al. Spiral architecture of the Hsp104 disaggregase reveals the basis for polypeptide translocation. Nat. Struct. Mol. Biol. 23, 830–837 (2016).

20. Han, H. & Hill, C. P. Structure and mechanism of the ESCRT pathway AAA+ ATPase Vps4. Biochem. Soc. Trans. https://doi.org/10.1042/BST20180260 (2018).

21. Monroe, N., Han, H., Shen, P. S., Sundquist, W. I. & Hill, C. P. Structural basis of protein translocation by the Vps4-Vta1 AAA ATPase. Elife 6, https://doi.org/10.7554/elife.24487 (2017).

22. Sandate, C. R., Szyk, A., Zehr, E. A., Lander, G. C. & Roll-Mecak, A. An allosteric network in spasticity couples multiple activities required for microtubule severing. Nat. Struct. Mol. Biol. 26, 671–678 (2019).

23. Lombard, L. M., Davis, M. D. & Rine, J. Maintenance of nucleosomal balance in cis by conserved AAA-ATPase Yta7. Genetics 199, 105–116 (2015).

24. Wang, T. et al. The histone chaperone FACT modulates nucleosome structure by tethering its components. Life Sci. Alliance 1, e20180107 (2018).

25. Chen, P. et al. Functions of FACT in breaking the nucleosome and maintaining its integrity at the single-nucleosome level. Mol. Cell 71, 284–293, e284 (2018).

26. Park, Y. J. & Luger, K. Histone chaperones in nucleosome eviction and histone exchange. Curr. Opin. Struct. Biol. 18, 282–289 (2008).

27. Gradolatto, A. et al. A noncanonical bromodomain in the AAA ATPase symmetric protein Yta7 directs chromosomal positioning and barrier chromatin activity. Mol. Cell Biol. 29, 4604–4611 (2009).

28. Gates, S. N., Lander, G. C. & Roll-Mecak, A. Ratchet-like poly peptide translocation mechanism of the AAA+ disaggregate Hsp104. Science 357, 273–279 (2017).

29. de la Peña, A. H., Goodall, E. A., Gates, S. N., Lander, G. C. & Martin, A. Substrate-engaged FACT in breaking the nucleosome and maintaining its integrity at the single-nucleosome level. Mol. Cell 71, 284–293, e284 (2018).

30. Mattiroli, F. et al. DNA-mediated association of two histone-compact boundaries of yeast Chromatin Assembly Factor-1 (CAF-1) drives tetrasome assembly in the wake of DNA replication. Elife 6, https://doi.org/10.7554/elife.22799 (2017).

31. Fadly, A. et al. Histone chaperone Rtt106 promotes nucleosome formation using (H3-H4)2 tetramers. J. Biol. Chem. 287, 10753–10760 (2012).

32. Zhoo, C. Y. et al. Regulation of Rvb1/Rvb2 by a domain within the INO80 chromatin remodeling complex implicates the Yeast Rvbs as protein assembly protein-unfolding machine. Mol. Cell 65, 858–867 (2015).

33. Luger, K., Rechtsteiner, T. J. & Richmond, T. J. Expression and purification of recombinant histones and nucleosome reconstitution. Methods Mol. Biol. 119, 1–16 (1999).

34. Lee, J. Y., Finkelstein, I. J., Arciszewska, L. K., Sherratt, D. J. & Greene, E. C. Single-molecule imaging of FisK translocation reveals mechanistic features of protein–protein collisions on DNA. Mol. Cell 54, 832–843 (2014).

35. Grant, T., Rohou, A. & Grigorieff, N. cis chemical cross-linking sites using LC-MS/MS and the xQuest/XProphet software pipeline. Nat. Protoc. 9, 120–137 (2014).

43. Leitner, A., Walzthoeni, T. & Aebersold, R. Lysine-specific chemical cross-linking of protein complexes and identification of cross-linking sites using LC-MS/MS and the xQuest/XProphet software pipeline. Nat. Protoc. 9, 120–137 (2014).