Histone H4 tail mediates allosteric regulation of nucleosome remodelling by linker DNA

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Imitation switch (ISWI)-family remodelling enzymes regulate access to genomic DNA by mobilizing nucleosomes1. These ATP-dependent chromatin remodelers promote heterochromatin formation and transcriptional silencing by generating regularly spaced nucleosome arrays2–5. The nucleosome-spacing activity arises from the dependence of nucleosome translocation on the length of extranucleosomal linker DNA6–10, but the underlying mechanism remains unclear. Here we study nucleosome remodelling by human ATP-dependent chromatin assembly and remodelling factor (ACF), an ISWI enzyme comprising a catalytic subunit, Snf2h, and an accessory subunit, Acf1 (refs 2, 11–13). We find that ACF senses linker DNA length through an interplay between its accessory and catalytic subunits mediated by the histone H4 tail of the nucleosome. Mutation of AutoN, an auto-inhibitory domain within Snf2h that bears sequence homology to the H4 tail4, abolishes the linker-length sensitivity in remodelling. Addition of exogenous H4-tail peptide or deletion of the nucleosomal H4 tail also diminishes the linker-length sensitivity. Moreover, Acf1 binds both the H4-tail peptide and DNA in an amino (N)-terminal domain dependent manner, and in the ACF-bound nucleosome, lengthening the linker DNA reduces the Acf1–H4 tail proximity. Deletion of the N-terminal portion of Acf1 (or its homologue in yeast) abolishes linker-length sensitivity in remodelling and leads to severe growth defects in vivo. Taken together, our results suggest a mechanism for nucleosome spacing where linker DNA sensing by Acf1 is allosterically transmitted to Snf2h through the H4 tail of the nucleosome. For nucleosomes with short linker DNA, Acf1 preferentially binds to the H4 tail, allowing AutoN to inhibit the ATPasen activity of Snf2h. As the linker DNA lengthens, Acf1 shifts its binding preference to the linker DNA, freeing the H4 tail to compete AutoN off the ATPasen and thereby activating ACF.

The packaging of DNA into nucleosomes presents a substantial energy barrier that restricts access to the genomic DNA13. ISWI-family remodellers use the energy from ATP hydrolysis to disrupt histone–DNA contacts and reposition nucleosomes1. The catalytic subunits of ISWI enzymes possess an SF2-like ATPasen that translocates DNA across the nucleosome1. The nucleosome translocation activity is further regulated by the accessory subunits of ISWI complexes6,10,16. Many ISWI remodellers exhibit a nucleosome-spacing activity2–5. Critical to this spacing activity are two features of the nucleosome that modulate the activity of ISWI remodellers: (1) the N-terminal tail of histone H4 (refs 8, 17–20) and (2) the length of the extranucleosomal linker DNA6–10. The unmodified H4 tail stimulates ISWI activity by relieving the autoinhibitory effect of the AutoN domain within the catalytic subunit22. H4 tail acetylation associated with transcriptionally active chromatin is thought to help prevent ISWI-induced nucleosome spacing at actively transcribed genes17–19. Regulation by the extranucleosomal linker DNA is responsible for generating the regularly spaced nucleosome arrays important for heterochromatin formation. Shortening the linker DNA reduces the remodelling activity of nucleosome-spacing ISWI enzymes6–10. As a result, nucleosomes are preferentially moved towards longer linkers to promote uniform spacing on nucleosome arrays. Interestingly, the catalytic activity of many ISWI-family enzymes is sensitive to linker DNA lengths up to approximately 60–70 base pairs (bp)6–10, consistent with the inter-nucleosome spacing of heterochromatin observed in human cells11. This linker-length sensing range substantially exceeds the binding footprint (20–30 bp) of the catalytic subunit22,23, whereas the accessory subunits of ISWI complexes can bind linker DNA as far as ~60 bp from the nucleosome edge24. However, it is unknown how accessory subunits communicate linker length information to the catalytic subunit to regulate remodelling activity. In this work, we investigate the mechanism underlying DNA linker-length sensitivity by a prototypical ISWI-family enzyme, human ACF.

To examine how linker DNA regulates nucleosome translocation by ACF, we reconstituted mononucleosomes with varying linker lengths (n = 20–78 bp) on the entry side but a constant exit-side linker length of 3 bp (Fig. 1a). We also constructed mononucleosomes with wild-type (WT) histone H4 and two H4 mutants: (1) H4 tail deletion (H4Δ1–19) and (2) H4 with K16A mutation (H4K16A). We refer to nucleosome constructs with the following nomenclature: [WT H4/H4Δ1–19/H4K16A, n bp] for nucleosomes with n bp of DNA on the entry side and an octamer containing WT H4, H4Δ1–19 or H4K16A. We detected ACF-catalysed nucleosome translocation using fluorescence resonance energy transfer (FRET) by labelling the end of the exit-side linker DNA with the FRET acceptor Cy5, and the histone H2A with the FRET donor Cy3 (Fig. 1a)24.

We first compared the remodelling kinetics of [WT H4, 78 bp], [WT H4, 40 bp], [WT H4, 20 bp] and [H4Δ1–19, 78 bp] nucleosomes using an ensemble FRET assay5. Upon addition of ACF and ATP, the FRET efficiency decreased as DNA was translocated towards the exit side (Fig. 1b and Extended Data Fig. 1a). As expected, the remodelling rate decreased as the linker DNA was shortened and deletion of the H4 tail drastically reduced the remodelling activity (Fig. 1b).

To identify which step(s) of the remodelling process are regulated, we monitored the remodelling of individual nucleosomes using single-molecule FRET24,25. Single-nucleosome remodelling traces featured incremental translocation of DNA to the exit side interrupted by kinetic pauses (Fig. 1c). The first pause occurred after ~7 bp of DNA translocation and the second pause occurred after an additional ~3 bp of translocation (Extended Data Fig. 2a, b), consistent with previous findings24,26. Moreover, the step sizes did not change with linker DNA length or histone H4 modification (Extended Data Fig. 2a, b). We divided the remodelling time trace into two translocation phases (T1, T2), during which the FRET efficiency decreased, and two pause phases (P1, P2), during which the FRET value remained constant (Fig. 1c). Notably, the DNA translocation rates between pauses did not change, whereas the pause-phase exit rates decreased dramatically when the linker DNA was shortened (Fig. 1d and Extended Data Fig. 2c). Moreover, the dependence of remodelling kinetics on entry-side linker lengths of mononucleosomes was quantitatively similar to the dependence on inter-nucleosome linker lengths up to approximately 60–70 base pairs (bp)6–10, consistent with the inter-nucleosome spacing of heterochromatin observed in human cells11. This linker-length sensing range substantially exceeds the binding footprint (20–30 bp) of the catalytic subunit22,23, whereas the accessory subunits of ISWI complexes can bind linker DNA as far as ~60 bp from the nucleosome edge24. However, it is unknown how accessory subunits communicate linker length information to the catalytic subunit to regulate remodelling activity. In this work, we investigate the mechanism underlying DNA linker-length sensitivity by a prototypical ISWI-family enzyme, human ACF.
lengths of dinucleosomes (Extended Data Fig. 3), validating the use of mononucleosomes as a model system to study linker-length sensitivity. Interestingly, the H4 tail appeared to regulate the same phase of the remodelling process as the linker DNA (Fig. 1e). The H4K16A mutation and H4 tail deletion (H4Δ1–19) decreased the pause-phase exit rate by approximately 2- and 20-fold, respectively (Fig. 1e). In contrast, neither modification had any appreciable effect on the translocation rates between pauses (Fig. 1e).

The above results indicate that both linker DNA and the H4 tail regulate the remodelling rate by changing the duration of pause phases, suggesting that these nucleosome features may impinge on an inhibitory mechanism that prevents the initiation of the DNA translocation phases. It has been shown that although the ISWI ATPase domain can translocate nucleosomes autonomously, the catalytic subunit contains two well-conserved autoregulatory domains, AutoN and NegC, which inhibit ATP hydrolysis and its coupling to DNA translocation, respectively. The AutoN inhibition can be relieved by the H4 tail whereas the NegC inhibition can be relieved by binding of the HAND-SANT-SLIDE module to linker DNA. Could the regulation of remodelling by linker DNA length occur through these inhibitory domains?

To address this question, we first examined the role of the NegC domain. Surprisingly, deletion of the NegC domain in the ACF complex (ΔNegC ACF) did not substantially affect the dependence of remodelling kinetics on linker DNA lengths ranging from 20 to 78 bp (Fig. 2a–c and Extended Data Fig. 4a). Removing the H4 tail dramatically reduced the remodel-ling rate of nucleosomes lacking the H4 tail, but also completely abolished the linker-length dependence of remodelling by specifically increasing the remodelling rate of short-linker nucleosomes (Fig. 3b, c and Extended Data Fig. 6). These results suggest an essential role for AutoN in linker length sensing by the ACF complex. Since AutoN competes with the H4 tail for binding to the ATPase, we considered the possibility that this competition is involved in sensing linker DNA length and hypothesized that the H4 tail is only available to compete AutoN off the ATPase when the linker DNA is sufficiently increased the remodelling rate of nucleosomes lacking the H4 tail, but also completely abolished the linker-length dependence of remodelling by specifically increasing the remodelling rate of short-linker nucleosomes (Fig. 3b, c and Extended Data Fig. 6). These results suggest an essential role for AutoN in linker length sensing by the ACF complex.

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Figure 3 | The AutoN domain of Snf2h and the nucleosomal H4 tail are important for linker DNA length sensing by the ACF complex. a, Domain architecture of WT and AutoN-2RA (R142A and R144A) Snf2h. b, Ensemble remodelling time courses of [WT H4, 78 bp], [WT H4, 40 bp] and [H4Δ1–19, 78 bp] nucleosomes by 40 nM WT ACF (black/grey lines, duplicated from Fig. 1b) and AutoN-2RA ACF (blue/cyan symbols) at 5 μM ATP. c, Dependence of the pause-phase exit rate on the linker DNA length and H4 tail for WT (black) or AutoN-2RA ACF (blue/cyan). *Too slow to be measured. d, Effect of the exogenously added H4-tail peptide on the pause-phase exit rates during remodelling by WT ACF. e, Pause-phase exit rates of nucleosomes lacking the H4 tail during remodelling by WT ACF. In c–e, [ACF] = 10 nM and [ATP] = 20 μM, except that 2 mM of ATP was used in e to make the pause exit rates measurable for nucleosomes lacking the H4 tail. Data are mean ± s.e.m. from at least 100 (c, d) or at least 50 (e) individual nucleosome remodelling traces from three independent experiments.

long. Consistent with this hypothesis, adding exogenous H4-tail peptide, which should help compete AutoN off the ATPase when the nucleosomal H4 tail is unavailable, specifically increased the remodelling rate of short-linker nucleosome ([WT H4, 40 bp]) by WT ACF (Fig. 3d). Furthermore, deletion of the nucleosomal H4 tail, in addition to slowing down remodelling, abolished the dependence of remodelling rate on linker DNA length (Fig. 3e). These results indicate that the H4 tail is indeed involved in linker DNA sensing.

Because the catalytic subunits of ISW1-family enzymes only interact with ~20–30 bp of extranucleosomal DNA, the linker-length sensitivity of ACF cannot be accounted for by the catalytic subunit alone. Our findings raise the intriguing possibility of a linker-length sensing mechanism where the accessory subunit Acf1 interacts with the H4 tail in a linker-length-dependent manner, which modulates the H4 tail availability for competing with AutoN. To test this possibility, we generated two Acf1 mutants, ΔC-term Acf1 and AN-term Acf1, in which 134 residues at the carboxy (C) terminus or 371 residues at the N terminus were deleted, respectively (Extended Data Fig. 7a and Fig. 4a). Because the central region of Acf1 required for Snf2h binding was not deleted, both mutants were able to form complexes with Snf2h, which are referred to as ΔC-term and AN-term ACF (Extended Data Fig. 4b).

We first probed which region of Acf1 interacts with the H4 tail by comparing the binding affinities of WT, ΔC-term and AN-term Acf1 for the H4-tail peptide using a fluorescence anisotropy assay. Interestingly, WT Acf1 exhibited specific, nanomolar affinity for the H4-tail peptide (Fig. 4b and Extended Data Fig. 7b) that was not substantially altered upon deletion of the C-terminal region (Extended Data Fig. 7c), but was completely lost upon deletion of the N-terminal portion (Fig. 4b). These results indicate that Acf1 interacts with the H4 tail probably through its N-terminal region. Acf1 also bound double-stranded DNA and deletion of the N-terminal region abolished this interaction too (Extended Data Fig. 8), consistent with the previous finding that the WAC motif within the N-terminal region is important for binding of ACF to the linker DNA28. Given the distinct properties of DNA and the H4 tail, their specific binding interfaces within Acf1 N-term are probably distinct.

Next, we investigated nucleosome remodelling by the ΔC-term and AN-term ACF complexes. Notably, the ΔC-term mutation did not substantially alter the dependence of remodelling kinetics on linker DNA length (Extended Data Fig. 7d), whereas the linker-length sensitivity was eliminated in the AN-term ACF complex (Fig. 4c, d). This finding is consistent with the specific affinity of Acf1 N-term for the H4 tail (Fig. 4b). Furthermore, if the loss of linker-length sensitivity was simply a result of losing the linker DNA binding affinity of Acf1, AN-term ACF should demonstrate inefficient remodelling for all linker DNA lengths. Instead, AN-term ACF remodelled both short- and long-linker nucleosomes at rates close to the rate with which WT ACF remodelled long-linker nucleosomes (Fig. 4c, d), suggesting that deletion of Acf1 N-term disabled a mechanism that inhibits remodelling at short linker lengths. AN-term ACF also maintained the H4-tail requirement in remodelling (Fig. 4c).

Since Acf1 has affinity to both DNA and the H4 tail, a plausible interpretation of the above observations is that the nucleosomal linker DNA and H4 tail are in competition for binding to the N-terminal region of Acf1 and that this competition is modulated by the length of the linker DNA. Only when the linker is sufficiently short does Acf1 preferentially bind to the H4 tail, making it unavailable to compete with the inhibitory AutoN. Deletion of Acf1 N-term diminishes the Acf1-H4 tail interaction such that the H4 tail is equally available to activate the ATPase at both short and long linker DNA lengths. We therefore probed the linker-length dependence of the Acf1-H4 tail proximity in ACF-bound nucleosomes featuring a cysteine-reactive crosslinker on the H4 tail. Specific H4-Acf1 crosslinking product was clearly observed as a band with reduced electrophoretic mobility compared with non-crosslinked Acf1 (Fig. 4e and Extended Data Fig. 9). Remarkably, the Acf1-H4 crosslinking efficiency decreased substantially with increasing linker DNA length (Fig. 4e), supporting our hypothesis that the Acf1-H4 tail interaction is modulated by the linker DNA length. In contrast, the H4-Snf2h crosslinking efficiency did not change substantially with linker DNA length, probably because Snf2h remains sufficiently close to the H4 tail regardless of the linker DNA length, which allows crosslinking even when the H4 tail was not specifically bound to its binding pocket on Snf2h.

Finally, we tested the physiological importance of the N-terminal region of Acf1 by studying the role of its homologue in yeast29. Yeast ISW2 is functionally similar to ACF. It is composed of a catalytic subunit (Isw2) that is homologous to Snf2h and three accessory subunits (Itc1, Dpb4 and Dls1), among which Itc1 is homologous to Acf1. We generated three mutant yeast strains: (1) deletion of the entire itc1 gene and rescue-strain by deleting the remaining portion of itc1 that encodes the N-terminal region of Itc1 equivalent to Acf1 N-term (ΔItc1-Nterm) and (2) a rescue strain that was derived from the ΔItc1-Nterm strain by deleting the remaining portion of itc1 (rescue-ΔItc1). Both ΔItc1 and rescue-ΔItc1 showed growth rates similar to that of the WT strain (Fig. 4f), consistent with previous observations29. In contrast, the ΔItc1-Nterm strain displayed dramatically slower growth (Fig. 4f), consistent with an aberrant chromatin misregulation phenotype.

Taken together, our results suggest a nucleosome-spacing mechanism for ACF in which the linker DNA length is sensed by the Acf1 accessory subunit and allosterically transmitted to the Snf2h catalytic subunit through the H4 tail of the nucleosome (Fig. 4g). Acf1 and the AutoN domain of Snf2h function collectively in DNA linker-length sensing. When the linker DNA is short, Acf1 preferentially binds to and sequesters the H4 tail, making it unavailable to compete its sequence homologue, AutoN, off the ATPase. Hence, the ATPase activity is inhibited by AutoN.
the linker DNA length increases, Acf1 shifts its binding preference to the linker DNA and releases the H4 tail, allowing it to compete AutoN off the ATPase and activate ACF. This competition between the H4 tail and linker DNA for Acf1 binding probably involves the N-terminal region of Acf1. It is interesting to note that linker DNA sensing occurs during the pause phases when the ATPase domain is not actively translocating DNA, suggesting that AutoN engages the ATPase domain during the pauses. To exit the pauses, the H4 tail is required to relieve the inhibitory effect of AutoN. The re-engagement of AutoN with the ATPase domain after each translocation phase would give ACF an opportunity to periodically sense the linker DNA length. Such frequent sensing may allow a more efficient nucleosome spacing, as previously hypothesized6. The linker DNA and the H4 tail are two important substrate features that regulate nucleosome remodelling by ISWI-family enzymes, the former enabling uniform nucleosome spacing for heterochromatin formation and the latter specifying regions of chromatin for silencing. Our results now reveal an unexpected convergence of the regulatory pathways defined by these two distinct nucleosome features.

**METHODS SUMMARY**

Detailed descriptions of nucleosome and ACF preparation, as well as single-molecule and ensemble FRET, fluorescence anisotropy, protein crosslinking and yeast experiments, are described in Methods. Briefly, various nucleosome constructs were reconstituted using Cy3-labelled histone octamers and Cy5-labelled DNA with a biotin moiety for surface anchoring. DNA was generated by PCR or by annealing and ligating a set of overlapping, complementary oligonucleotides (Extended Data Fig. 10). Histone octamer, nucleosomes, Acf1, Snf2h and ACF complexes were reconstituted and purified as described previously6,7,24. Mutant yeast strains were generated in the ITC1 gene (itc1-Nterm). Top row: WT. Second row: the coding sequence of the N-terminal region of Acf1 is deleted (Δitc1). Third row: the coding sequence of the N-terminal region of Acf1 is deleted (Δitc1-Nterm). Bottom row: the remaining portion of itc1 gene is deleted (Δitc1). One representative of three independent growth experiments is shown. g, Model for linker DNA length sensing by the ACF complex. DNA: grey lines; histone octamer: beige cylinders; Snf2h: blue/cyan; Acf1: green. The ATPase domain of Snf2h is depicted as a cyan sphere and labelled ‘On’ when active and ‘Off’ when inactive.
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Author Contributions W.L.H., S.D. and X.Z. designed the experiments, with input from B.T.H. W.L.H. and S.D. performed the experiments and data analysis. B.T.H helped prepare the histones and nucleosomes. S.D., W.L.H. and X.Z. wrote the paper, with input from B.T.H. X.Z. oversaw the project.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to X.Z. (zhuang@chemistry.harvard.edu).
METHODS

DNA constructs. Double-stranded (ds) DNA constructs containing the 601 nucleosome positioning sequence22, varying DNA linker lengths on the entry side of the nucleosome, 3 bp linker DNA on the exit side of the nucleosome, a FRET acceptor Cy5 attached to the 5′-end of the exit-side linker DNA and a biotin moiety attached to the 5′-end of the entry-side linker DNA were generated by PCR and purified by PAGE. The dsDNA constructs with an additional 38 nucleotide (nt) single-stranded (ss) DNA spacer used for single-molecule mononucleosome remodelling experiments were created by annealing and ligating a set of overlapping, complementary oligonucleotides. The 38 nt ssDNA spacer was used to prevent surface perturbation in remodelling. These high-performance liquid chromatography (HPLC)-purified oligonucleotides (Integrated DNA Technologies) were mixed at equimolar concentrations in 50 mM Tris pH 8.0, 100 mM KCl, 1 mM EDTA and annealed with a temperature ramp (95–3 °C), ligated with T4 DNA ligase (New England Biolabs) and purified by PAGE. Successful ligation was confirmed by denaturing PAGE. To make dinucleosomes, DNA constructs for each mononucleosome were first created using the above annel-ligate approach. In this case, the 38 nt ssDNA linker DNA. Top strand: 5′-GC57CGCTGCAATCCCTTTAGGTTTACCGGGCCTTGCCCGGAGAGGTTCAGG-3′; 5′-TCTTACGGTGACGTGCAGCACCACCTTTAC-3′.

For site-specific labelling of histone H2A, a cysteine substitution was introduced at residue 120 (K120C) as described previously14. Lyophilized H2A was dissolved in labelling buffer (7 M guanidine-HCl, 20 mM Tris pH 7.0, 5 mM Na-Erta, 1.25 mM TCEP) and incubated for 2 h in the dark. Cy3-maleimide was dissolved in DMSO and added to the reaction to a final concentration of 2.5 mM. After incubating for 3 h in the dark, the reaction was quenched by adding 80 mM β-mercaptoethanol. The labelled H2A was dialysed three times against dialysis buffer (7 M guanidine HCl, 20 mM Tris pH 7.0, 1 mM DTT) in a 7K MWCO dialysis cassette (Pierce) and purified by PAGE.

To reconstitute histone octamer, one lyophilized core histone was dissolved in unfolding buffer (7 M guanidine-HCl, 20 mM Tris-HCl pH 7.5, 10 mM DTT), mixed and added to the reaction to a final concentration of 2.5 mM. After incubating for 3 h in the dark, the reaction was quenched by adding 80 mM β-mercaptoethanol. The labelled H2A was dialysed three times against dialysis buffer (7 M guanidine HCl, 20 mM Tris pH 7.0, 1 mM DTT) in a 7K MWCO dialysis cassette (Pierce) and purified by PAGE.

For making FRET-labelled nucleosomes, a ratio of approximately 1:1 of Cy3-labelled and unlabelled H2A mixture was used. Because each histone octamer contained two H2A subunits, this reconstitution yielded three distinct populations of Cy3-labelled nucleosomes: (1) with Cy3 attached only to the H2A subunit proximal to the FRET acceptor Cy5 on the DNA, (2) with Cy3 attached only to the H2A subunit distal to the Cy5 on the DNA and (3) with both H2A labelled by Cy3. These three labelling configurations yielded different FRET levels that could be clearly distinguished at the single-molecule level, as shown previously23. To maximize the dynamic range for single-molecule FRET measurements, we selected the population with the highest FRET value, which corresponded to nucleosomes with a single Cy3 on the proximal H2A, for further analysis. For ensemble FRET measurements, because the overall remodelling rate was derived from the decay curve of the acceptor signal, distinguishing the three populations was unnecessary. For crosslinking experiments, only unlabelled H2A was used.

Nucleosomes. Mononucleosomes were reconstituted from DNA and histone octamers at 4 °C (ref. 32). The nucleosome reconstitution reaction consisted of 2 M KCl, 20 mM Tris-HCl pH 7.5, 1 mM Na-Erta, 1.2 mM histone octamer, 1 mM DNA construct, 10 mM DTT and 0.5 mM benzanilide. The sample was injected into a pre-hydrated 7K MWCO dialysis cassette (Pierce) and subjected to salt gradient dialysis (2 M KCl to 250 mM KCl over 60 h) followed by incubation in TBS buffer (20 mM Tris-HCl pH 7.5, 1 mM EDTA, 1 mM DTT) for 8 h at 4 °C. Assembled nucleosomes were purified on a 10–30% glycerol gradient in 20 mM Tris-HCl pH 7.5, 1 mM Na-Erta, 0.1% Igepal CA-630. Fractions were analysed by PAGE (5% 0.5× TBE) and fractions containing pure and correctly assembled nucleosomes were pooled and concentrated with a 100K MWCO Amicon Ultra-0.5 mL centrifugal filter (Millipore) to ~30 μl final volume and stored at 4 °C. For the assay of nucleosomes used in crosslinking experiments, reducing agent was omitted.

Dinucleosomes were prepared by assembling each mononucleosome separately and then ligating these mononucleosomes (Extended Data Fig. 3a). The distal nucleosome further away from the surface-anchoring site was FRET-labelled in the same way as the mononucleosome constructs. The proximal unlabelled nucleosome closer to the surface-anchoring site featured 2 nt gaps at the super-helical location ± 2 (SHL ± 2) sites to prevent translocation and biotin at the DNA end for surface-anchoring. Each ligation reaction contained approximately 1 pmol of total nucleosome, 3 pmol of FRET-labelled nucleosome over the proximal unlabelled nucleosome, 1 × T4 DNA ligase buffer (New England Biolabs) supplemented with 10 mM KCl, 0.1% Igepal CA-630 and 2000 units of T4 DNA ligase (New England Biolabs) in a final volume of 20 μl. Ligation was allowed to proceed at room temperature for 1 h followed by the addition of EDTA and glycerol to final concentrations of 1 mM and 20%, respectively, and storage at 4 °C. Purification was unnecessary because only the constructs that contained both distal FRET-labelled and proximal nucleosomes with a biotin moiety could be anchored to the surface and generate a FRET signal.

ACF. To produce isolated Snf2h-Flag or isolated Acf1-Flag, these proteins were overexpressed in S9 cells using a baculovirus expression system (Kinnakeet Bioscience Technology). To produce ACF complexes, Snf2h and Acf1-Flag were co-expressed in S9 cells. Nuclear extraction was performed as described previously24,25. Nuclear extract was fortified with 0.5 mM benzamidine, 60 μg ml−1 TLCK and 1× Roche Complete Protease Inhibitor Cocktail and then purified by M2-antiflag affinity chromatography (Flag-anti M2 beads) and Flag peptide (Sigma-Aldrich), as described previously26. Specifically, the Flag-tagged subunit or complex was bound to M2 beads, and the beads were washed several times with wash buffer containing 20 mM HEPES, pH 7.9, 20% glycerol, 0.2 mM EDTA, 1 mM benzamidine, 1 mM DTT, Roche EDTA-free complete protease inhibitor and various concentrations of KCl (300, 150 and 100 mM sequentially). Protein was subsequently eluted with a 1 mMFLAG peptide solution in wash buffer with 100 mM KCl. Elution fractions were analysed by PAGE and fractions containing the isolated subunits or the intact ACF complex were pooled and concentrated with a 50K MWCO Amicon Ultra-15 mL centrifugal filter (Millipore). Concentrated ACF complexes, Snf2h subunit and Acf1 subunit

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were then aliquoted, flash frozen and stored at −80 °C in 20 mM HEPES, pH 7.9, 20% glycerol, 0.2 mM EDTA, 100 mM KCl and 1 mM DTT. To purify enzyme for crosslinking experiments, the complete protease inhibitor pills and DTT were omitted from the buffers during the affinity purification steps. Typical yields were ~50–100, 20–40 and 1–2 μg of ACF complexes, Snf2h subunit and Acf1 subunit, respectively, per litre of insect cell culture. Quantification was by SYPRO red staining and comparison with BSA standards. The Snf2h and Acf1 mutants were created by site-directed mutagenesis using the following primers. Auto-N-2A Snf2h (R14A, R144A): 5′-CTCTCTATGCCTGCAATACAGCAGAACGAGAAG-3′; 5′-TCCCTTGGCTTGCTACTGAAATCCGGTAATGCCAACACGGATG-3′. Acf1 (Δ679–700, insert SGSGS): 5′-GGTGGTGCCTCAGAAAAGCTTGAAGCTGTTGGCTTGCTCTGGTACG-3′; 5′-TCTGGTAATCTATGTTCAAGATTTCTGACTTGAATGCCATGATT-3′. Enzymes were translated towards the centre of the DNA, the FRET efficiency decreased, which resulted in a decrease in the Cy5 signal.

The ensemble nuclease remodelling assay was performed by diluting nuclease solutions in remodelling buffer (40 mM Tris pH 7.5, 12 mM HEPES pH 7.9, 60 mM KCl, 0.32 mM EDTA, 3 mM MgCl2, 100 μM ATP and 2 mM GTP) containing histone H4, immediately following the initial methionine. The other histone proteins used to prepare H4 nuclease solutions were WT H2A, WT H2B and H3C110A, which did not contain additional cytosine residues. The FRET-labelling reaction was done at room temperature for 1.5 h. Buffer exchange with wash buffer (20 mM HEPES pH 7.0, 1 mM EDTA, 10% glycerol, 10 mM KCl, 0.1% Igepal CA-630) used a 100K MWCO Amicon Ultra-0.5 mL centrifugal filter (Millipore) five times to remove unreacted crosslinker. ACF was then added to the BM(PEG)3-derivatized nuclease solutions to achieve a final enzyme concentration of ~200–400 nM and nucleosome concentration of 5 μM, and incubated for 2–3 h. Symmetrical nucleosomes with equal linker DNA lengths on both sides were used to produce data shown in Fig. 4e and Extended Data Fig. 9. Similar results were obtained from asymmetric nucleosomes with 3 bp linker DNA on the exit side and varying linker lengths on the entry side. Crosslinking products were analysed by SDS-PAGE (4–15%, 1× Tris/glycine/SDS) and SYPRO red-staining. Under each condition, the ACF-H4 crosslinked fraction was calculated as Acf1-H4/(Acf1-H4 + Acf1) × [Acf1-H4], where [Acf1-H4] and [Acf1] are the integrated intensities of the Acf1-H4 and Acf1 bands, respectively, and [Acf1-H4] is the relative histone amount obtained by dividing the histone band intensity in a given lane by the maximum histone band intensity in the gel. The Snf2h-H4 crosslinked fraction was obtained analogously. Normalization by the relative histone intensity in each lane compensated for any small variation in the nucleosome concentration used, even though efforts were made to maintain identical nucleosome concentrations for all conditions. For validation of the H4-crosslinking product, immunoblotting used a primary rabbit polyclonal λ-histone H4 antibody (Abcam ab10158, tested for Western Blotting applications by the ‘Abpromise’ guarantee) and a horseradish-peroxidase-conjugated secondary antibody, and H4 was detected by chemiluminescence (Healthcare ECL).

Yeast genetics experiments. Mutant yeast strains were generated in the BY4741 background (ATCC 4006733, MA; his3Δ1; leu2Δ0; met15Δ0; urs3Δ0). This strain lacks the ura3-1 gene that encodes an enzyme involved in uracil synthesis and includes equal linker DNA lengths on both sides. The ura3Δ1 strain was derived from BY4741 by replacing (through yeast transformation and integration by homologous recombination) the trc1 cassette with the trc1Δ cassette and selecting the ura3Δ1 trc1Δ strain and ura3Δ1 trc1Δ ura3Δ1 double mutant, respectively. Positive transformants were selected on media lacking uracil (uracil-dropout). The ura3Δ1 trc1Δ strain was generated by PCR from the pRS306 plasmid (primers used: taaacataggaagaagaagag cggatatcaatcctgggctctgctaccagc and atttggcagcggaagcttgctcgtgctgttccatcgtctggcactctgggttctggcactctggggttctggcact). The Δict1-1Nerm strain was derived from the Δict1Δ1 strain by replacing the YRΔ3 cassette with the Δict1Δ1 cassette and selecting the Δict1Δ1 strain and Δict1Δ1 ura3Δ1 double mutant. The Δict1Δ1 ura3Δ1 strain was then selected on media lacking uracil (uracil-dropout). The Δict1Δ1 strain was generated by PCR from the pRS306 plasmid (primers used: Δict1Δ1 strain and Δict1Δ1 ura3Δ1 strain). Nonlinear curve fitting of the fluorescence anisotropy data used Matlab, and 95%
confidence intervals for fit parameters were obtained using the ‘confint’ routine. Experimental sample sizes as indicated in the figure captions gave the reported s.e.m. values that were sufficiently low to allow meaningful interpretation of the data.

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Extended Data Figure 1 | Control experiments to test the effect of ATP and surface-anchoring on nucleosome remodelling. a, Ensemble remodelling time courses of [WT H4, 78 bp] nucleosomes by 10 nM ACF with 5 μM ATP (filled symbols) or without ATP (open symbols). Nucleosome translocation was monitored by the emission intensity of the FRET acceptor Cy5 under constant 532 nm illumination that excited the FRET donor Cy3. b, Comparison of the average remodelling kinetics for surface-anchored [WT H4, 40 bp] nucleosomes (measured by the single-molecule assay, more than 250 nucleosomes) and freely diffusing [WT H4, 40 bp] nucleosomes in solution (measured by the ensemble assay). [ACF] = 5 nM and [ATP] = 20 μM.
Extended Data Figure 2 | Translocation step sizes and dwell time distributions of translocation and pause phases during nucleosome remodelling. 

**a**, Histogram of FRET levels for the initial position and pause positions of [WT H4, 40 bp] nucleosomes upon remodelling by ACF. The histogram was fitted by multiple Gaussian peaks (black line) and the peak values were used to compute the average translocation distances between pauses. The translocation distances could be quantified using a calibration curve of FRET efficiency versus exit-side linker DNA length\(^{24,26}\), yielding a 7.0 bp step size between the initial position and the first pause and a 3.4 bp step size between the first pause and the second pause. 

**b**, Step sizes for various mononucleosomes and dinucleosomes. The dinucleosome constructs, [WT H4, 40 bp, WT H4] and [WT H4, 78 bp, WT H4], are each composed of one FRET-labelled nucleosome and one unlabelled nucleosome, spaced by 40 bp and 78 bp of internucleosomal linker DNA, respectively. The flanking linker DNA is 3 bp on the side of the FRET-labelled nucleosome and 40 bp on the side of the unlabelled nucleosome. The unlabelled nucleosome contains 2-nt ssDNA gaps at the SHL± two sites to prevent translocation. Data are mean ± s.e.m. derived from at least 100 remodelling traces from three independent experiments. 

**c**, Dwell-time distributions for the first translocation phase, \(t_T\), and the first pause phase, \(t_P\), for nucleosomes with different lengths of linker DNA. [ACF] = 10 nM and [ATP] = 20 μM.
Extended Data Figure 3 | DNA linker-length sensing by ACF is quantitatively similar for mononucleosomes and dinucleosomes. a, The dinucleosomes contain a distal FRET-labelled nucleosome and a proximal unlabelled nucleosome connected by \( n \) bp of internucleosomal linker DNA. The flanking linker DNA is 3 bp and 40 bp on the side of the FRET-labelled and the unlabelled nucleosome, respectively. To facilitate the study of translocation of the FRET-labelled nucleosome, we placed 2-nt ssDNA gaps at the SHL ± two sites of the proximal nucleosome to prevent its repositioning. b, Comparison of translocation and pause-phase exit rates for mononucleosomes (filled bars) and dinucleosomes (hashed bars) with 40 bp and 78 bp linker lengths. [ACF] = 10 nM and [ATP] = 20 \( \mu \)M. Data are mean ± s.e.m. derived from at least 100 individual nucleosome remodelling traces from three independent experiments.
Extended Data Figure 4 | SDS–PAGE analysis of WT, ΔNegC, AutoN-2RA, ΔN-term and ΔC-term ACF complexes. a, WT Snf2h, ΔNegC Snf2h or AutoN-2RA Snf2h was co-expressed with Acf1-Flag in Sf9 insect cells and purified by affinity chromatography. b, ΔN-term Acf1 or ΔC-term Acf1 was co-expressed with Snf2h-Flag and purified by affinity chromatography. The presence of both Acf1 and Snf2h in each case indicated that WT SNF2h, ΔNegC SNF2h and AutoN-2RA SNF2h can all form complexes with Acf1 and that ΔN-term Acf1 and ΔC-term Acf1 can both form complexes with Snf2h.
Extended Data Figure 5 | Short-range linker-length sensing by the isolated Snf2h catalytic subunit requires the NegC domain. Ensemble remodelling time courses of [WT H4, 78 bp], [WT H4, 40 bp] and [WT H4, 20 bp] nucleosomes by 130 nM WT (a) or ΔNegC Snf2h (b) at 2 mM ATP.
Extended Data Figure 6 | Ensemble remodelling time courses of nucleosomes by WT and AutoN-2RA ACF at two different enzyme concentrations. 

**a.** Remodelling of [WT H4, 78 bp] and [WT H4, 40 bp] nucleosomes by 40 nM (top) and 10 nM (bottom) WT ACF.

**b.** Remodelling of [WT H4, 78 bp] and [WT H4, 40 bp] nucleosomes by 40 nM (top) and 10 nM (bottom) AutoN-2RA ACF.
Extended Data Figure 7 | The C-terminal region of Acf1 is not required for specific binding to the H4 tail or for linker-length sensing by the ACF complex. 

**a**, Domain maps of WT and ΔC-term Acf1 (residues 1423–1556 deleted). 

**b**, Fluorescence anisotropy of TMR-labelled WT or 2RA H4-tail peptide in the presence of varying amounts of WT Acf1. In the 2RA H4-tail peptide, two charged arginines in the basic patch of the H4-tail peptide corresponding to the 2RA mutation in AutoN were replaced with alanines. $K_d$ for the 2RA H4-tail peptide (41 ± 29 nM) is substantially higher than that of the WT H4-tail peptide (3 ± 9 nM). Data are presented as mean ± s.e.m. (error bars, 95% confidence intervals, $n = 3$ three independent titration experiments).

When excess unlabelled H4-tail peptide was added to compete the TMR-labelled peptide off Acf1, the fluorescence anisotropy was reduced to the background level observed in the absence of Acf1. 

**c**, Fluorescence anisotropy of TMR-labelled H4-tail peptide in the presence of varying amounts of WT or ΔC-term Acf1. The measured $K_d$ for ΔC-term Acf1 is 2 ± 7 nM, which is similar to that for WT Acf1 (3 ± 9 nM). Data are presented as mean ± s.e.m. (error bars, 95% confidence intervals, $n = 3$ independent titration experiments).

**d**, Ensemble remodelling time courses of [WT H4, 78 bp], [WT H4, 40 bp] and [H4Δ1–19, 78 bp] nucleosomes by 40 nM WT (black/grey lines) and ΔC-term ACF (purple/light purple symbols) at 5 μM ATP.
Extended Data Figure 8 | Binding of dsDNA to Acf1 depends on its N-terminal region. Electrophoretic mobility of dsDNA (225 bp, 8 nM) in the presence or absence of 22 nM WT or ΔN-term Acf1. As a comparison, lanes 2 and 4 show Acf1 samples without the dsDNA.
Extended Data Figure 9 | α-histone H4 immunoblot analysis validates the formation of the Acf1-H4 crosslinked product. a, Left: SDS–PAGE analysis of samples containing ACF alone or ACF with nucleosomes (20 bp linker DNA) that do not possess the cysteine-reactive crosslinker on the H4 tail. Both samples yield two distinct bands corresponding to the Acf1 and Snf2h subunits (180 kDa and 122 kDa, respectively). Additionally, histone bands at low molecular masses are present in the lane for the sample containing nucleosomes. Right: corresponding immunoblot using α-H4 antibody. In the presence of nucleosomes without crosslinker, a single H4 band is visible at approximately 11 kDa corresponding to the histone itself. b, Top: incubation of ACF and nucleosomes that contain a crosslinker at the H4 tail yield Acf1-H4 and Snf2h-H4 crosslinking bands. These bands are absent for ACF without addition of nucleosomes (‘−nucleosomes’) or upon addition of nucleosomes without a crosslinker (‘+ nucleosomes (−crosslinker)’). Proteolytic degradation of Acf1 gave rise to a fainter band immediately below Acf1. Bottom: α-histone H4 immunoblotting reveals specific Acf1-H4 and Snf2h-H4 bands that are absent for ACF without addition of nucleosomes or upon addition of nucleosomes without a crosslinker.
Extended Data Figure 10 | DNA constructs used for mononucleosomes and dinucleosomes. The 601 nucleosome positioning sequence is shown in green (601* represents the introduction of 2 nt gaps at nucleotides 53 and 54 in the top and bottom strands of the 601 positioning sequence, respectively). For mononucleosome DNA constructs, the ssDNA spacer used to circumvent surface effects in single-molecule FRET measurements is underlined. Constructs referred to as 'mononucleosome [or dinucleosome] with n bp linker DNA' were used in single-molecule and bulk remodelling experiments. For ensemble remodelling experiments with mononucleosomes, the ssDNA spacer was omitted without any appreciable change in the overall remodelling kinetics. Constructs referred to as 'symmetric mononucleosomes with n bp linker DNA' were used in crosslinking experiments. Asymmetric constructs with the same linker length on one side of the nucleosome but only 3 bp of linker DNA on the other side displayed quantitatively similar crosslinking behaviour.