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Regulation of ISWI involves inhibitory modules antagonized by nucleosomal epitopes

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Abstract

Chromatin remodeling complexes (CRCs) mobilize nucleosomes to mediate the access of DNA-binding factors to their sites *in vivo*. These CRCs contain a catalytic subunit that bears an ATPase/DNA translocase domain, and flanking regions that bind nucleosomal epitopes[1]. A central question is whether and how these flanking regions regulate ATP hydrolysis or the coupling of hydrolysis to DNA translocation, to affect nucleosome sliding efficiency. ISWIfamily CRCs contain ISWI[2], which utilizes its ATPase/DNA translocase domain to pump DNA around the histone octamer to enable sliding[3-7]_ENREF_13. ISWI is positively regulated by two 'activating' nucleosomal epitopes: the 'basic patch' on the H4 tail, and extranucleosomal (linker) DNA[8-13]. Previous work defined the HSS domain in the ISWI C-terminus that binds linker DNA, needed for ISWI activity[14,15]. Here, we define two new, conserved, and separate regulatory regions on *Drosophila* ISWI, AutoN and NegC, that negatively regulate ATP hydrolysis (AutoN) or the coupling of ATP hydrolysis to productive DNA translocation (NegC). Rather than 'activating', the two aforementioned nucleosomal epitopes actually inhibit the negative regulation of AutoN and NegC. Remarkably, mutation/removal of AutoN and NegC enables significant nucleosome sliding without the H4 'basic patch' or extranucleosomal DNA, or the HSS domain – converting ISWI to biochemical attributes of SWI/SNF-family ATPases. Thus, the ISWI ATPase catalytic core is an intrinsically-active DNA translocase which conducts nucleosome sliding, onto which selective 'inhibition-of-inhibition' modules are placed, to help ensure that remodeling occurs only in the presence of proper nucleosomal epitopes. This supports a general concept for the specialization of chromatin remodeling ATPases, where specific regulatory modules adapt an ancient active DNA translocase to conduct particular tasks only on the appropriate chromatin landscape.

ISWI is the catalytic subunit of a set of CRCs with orthologs in all eukaryotes[1] (Fig.1). To define how ISWI is intrinsically regulated, we undertook an extensive structure-function analysis of the *Drosophila* ISWI ATPase, and its regulation by the histone H4 tail and extranucleosomal DNA. We recognized in the amino terminus of ISWI a region similar to the 'basic patch'[10] of the histone H4 tail (Fig. 1a), and conserved in eukaryotes (Fig. 1b). To test function, we replaced either (R91A or R93A) or both arginines (2RA) with alanine.

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Nucleosome sliding by wild-type ISWI ($ISWI^{wt}$) normally requires nucleosomes with intact H4 tails[8,9] and the presence of extranucleosomal DNA[11,12], however $ISWI^{wt}$ ATPase activity can be elicited by combining DNA and H4 tail peptide bearing the basic patch (Fig. 1c)[10]. Remarkably, the $ISWI^{2RA}$ derivative displayed ~3-fold higher ATPase activity than $ISWI^{wt}$ and was independent of H4 epitopes (Fig. 1c). For extended nucleosomes (200bp, 601 sequence, centrally-positioned), loss of either the basic patch (A17, A19) or the H4 tail (g4) markedly diminished (5-fold) $ISWI^{wt}$ activity, whereas $ISWI^{2RA}$ was only modestly attenuated, retaining ~63% activity and ~9-fold higher activity than $ISWI^{wt}$ (Fig. 1d). Notably, $ISWI^{R91A}$ and $ISWI^{R93A}$ resemble moderate and weak, respectively, gainof-function derivatives; as their ATPase activities increase, their dependence on the H4 tail decreases (Supplementary Fig. 1). Thus, $ISWI^{2RA}$ greatly activates ATPase activity, and renders the enzyme largely independent of the H4 tail, defining an N-terminal autoinhibitory region (AutoN) with similarity to the H4 tail (see Fig. 4b).

Remodeler ATPases are SF2-family[16] DNA translocases that couple DNA translocation to ATP hydrolysis[3-7,17-19]_ENREF_2, wherein the two RecA-like protein lobes of the ATPase domain sequentially grip and release the DNA backbone, typically moving a small number of base pairs per ATP hydrolysis[20,21]_ENREF_2. 'Coupling' refers to how efficiently ATP hydrolysis results in productive DNA translocation. Uncoupling by mutation is well documented[22,23]_ENREF_24 but of unknown significance - the key question is whether and how coupling is regulated by nucleosomal epitopes. To monitor coupling, we employed a 'tethered translocation' system, which uses plasmid supercoiling to quantify DNA translocation by ISWI (Supplementary Fig. 2). Remarkably, DNA translocation by $ISWI^{wt}$ was greatly enhanced by H4 tail peptide, whereas $ISWI^{2RA}$ showed robust DNA translocation without peptide (Fig. 2a), consistent with our ATPase results (Fig. 1c,d).

Nucleosome sliding was monitored by achieving an equilibrium between end- and center-positioned octamers, from an initial center-positioned population (Fig. 2b). $ISWI^{2RA}$ yielded an end↔center equilibrium much faster than $ISWI^{wt}$; $ISWI^{2RA}$ shifted in 30″ the amount shifted in 30′ by $ISWI^{wt}$ (Fig. 2b). $ISWI^{wt}$ required the full H4 tail for *in vitro* sliding (shown previously)[8], and here we establish the importance of the basic patch (Fig. 2c, upper panels). Remarkably, $ISWI^{2RA}$ partially bypassed mutations in the H4 basic patch, enabling sliding, but only weakly bypassed the full H4 tail (Fig. 2c, lower panels). This suggested an additional H4 epitope (beyond the basic patch) needed for coupling high ATPase activity to efficient sliding, and an extensive set of experiments involving peptides and nucleosomes bearing mutations in the H4 tail revealed a major contribution from H4K12 in promoting coupling (Supplementary Fig. 3).

We next examined core nucleosomes (147 bp, 601 sequence). Supporting previous work[11], $ISWI^{wt}$ activity required both extranucleosomal DNA and H4 tail epitopes (Fig. 1d, 2d). Interestingly, with $ISWI^{2RA}$, both ATPase and sliding remained efficient on core nucleosomes (Fig. 1d, 2d), establishing independence from extranucleosomal DNA. However, omitting the H4 tail greatly reduced sliding but not ATPase activity with $ISWI^{2RA}$ (Fig. 1d, 2d). Taken together, AutoN functions as an autoinhibitory region, that when mutated (in $ISWI^{2RA}$) largely bypasses the H4 tail for ATPase stimulation, but retains reliance on the H4 tail to 'couple' that ATP hydrolysis to productive DNA translocation.

One simple model is that AutoN binds to and holds the ATPase domain in an inactive conformation, with the H4 tail competing for part of that surface; the binding of the H4 tail displaces AutoN and enables a conformational change that activates ISWI ATPase activity – an effect mimicked by the ISWI^{2RA} mutation. Here, the ATPase activity of our derivative series (2RA>R91A>R93A) was the reciprocal of H4 tail stimulation (Supplementary Fig. 1), consistent with competition/antagonism for a region on ISWI involving the H4 tail and AutoN. Consistent with this model, a peptide from the AutoN region of ISWI^{wt} (K76-E98) does not activate ISWI (Fig. 1c).

Previous work defined a HAND-SANT-SLIDE domain (HSS) present in the C-terminus of ISWI (and related Chd1) that binds extranucleosomal DNA, needed for ISWI and Chd1 remodeling activity[14,15,24]. However, our results with AutoN prompted a search for an analogous unknown region that prevents ATPase action unless the HSS is bound to extranucleosomal DNA. Mutiple approaches (alignments, protease mapping, structural modeling) yielded a candidate region (covering residues 617 to 648) immediately following the second of the two RecA-like ATPase lobes, which we term NegC (Fig. 3a, 3b). A structural model using Phyre^2 ([25]; Fig. 3b), revealed NegC as a 'C-terminal bridge' traversing from ATPase lobe #2 back to the ATPase lobe #1, crossing the key functional ATPase cleft. Furthermore, this structural model of NegC is consistent with crosslinking experiments of ISWI[26], and a recent crystal structure of Chd1[27]. Notably NegC is conserved only in remodelers bearing an HSS domain, and regulated by extranucleosomal DNA (ISWI and Chd1, not SWI/SNF), though NegC function is entirely unknown.

To test NegC function, we isolated three ISWI C-terminal truncation derivatives: ΔC697 (omitting the HSS), ΔC648 (further deletion), and ΔC617 (omitting NegC), as well as combinations involving 2RA/AutoN mutations. First, omitting C-terminal regions from ISWI^{wt} did not render them stimulatable by naked DNA (Fig. 3c). In contrast, both full-length ISWI^{2RA} and ISWI$^{2RA/\Delta C}$ derivatives were highly stimulated by DNA (Fig. 3c), showing that the ATPase domain itself has an intrinsic DNA-dependent ATPase activity which is held in check by the AutoN region (Fig. 4b). Notably, removal of the HSS domain (ΔC697 or ΔC648) prevented DNA translocation (Fig. 3d) while retaining high ATPase activity (Fig. 3c, for ISWI^{2RA}), demonstrating a lack of coupling. Remarkably, further truncation to ΔC617 (ISWI$^{2RA/\Delta C617}$) restored DNA translocation (Fig. 3d) - establishing that NegC negatively regulates the coupling of ATP hydrolysis to DNA translocation (Fig. 4b).

We then tested these ISWI$^{\Delta C}$ derivatives with core or extended nucleosomes that either contain or lack the H4 tail (Fig. 3e). As expected[14], omitting the HSS domain in ISWI$^{\Delta C697}$ (or ISWI$^{\Delta C648}$) greatly reduced sliding of extended nucleosomes (lanes 3, 4, 19, 20). Surprisingly, ISWI$^{2RA/\Delta C697}$ and ISWI$^{2RA/\Delta C648}$ displayed high ATPase activity, but completely failed to slide either core (lanes 23 and 24) or extended nucleosomes (lanes 7 and 8), mirroring the uncoupling of ATPase activity and DNA translocation above (Fig. 3c, d). Remarkably, and in keeping with our tethered translocation results, further removal of NegC (ΔC617) restored sliding activity, without an accompanying increase in ATPase activity. Restoration was moderate with ISWI$^{\Delta C617}$ (lane 2) and efficient with ISWI$^{2RA/\Delta C617}$ (lane 6) on extended nucleosomes. Similarly, with core nucleosomes, sliding

was low but detectable with ISWI$^{\Delta C617}$ (lane 18), and efficient with ISWI$^{2RA/\Delta C617}$ (lane 22). Thus, NegC is a new region that negatively regulates sliding via ATPase/translocation uncoupling. NegC is entirely separate from, and opposite in function to, characterized regions in other ATPases that promote sliding[23,28].

We then addressed how ISWI integrates the presence of nucleosomal epitopes. For ISWI^{wt}, omission of extranucleosomal DNA (lane 17) greatly impaired sliding whereas omission of the H4 tail (lane 9, or omitting both, lane 25) prevented sliding. With ISWI^{2RA}, loss of both the H4 tail and extranucleosomal DNA prevented sliding (lane 29), even though ATPase activity was high; comparable to ISWI^{wt} on extended nucleosomes (lane 1). However, ISWI^{2RA} could slide extended nucleosomes lacking the H4 tail (lane 13), or core nucleosomes bearing H4 tails (lane 21). Thus, both the H4 tail and extranucleosomal DNA contribute to coupling (Fig. 4b). Importantly, whereas ISWI$^{2RA/\Delta C697}$ and ISWI$^{2RA/\Delta C648}$ are unable to slide nucleosomes lacking the H4 tail (g4; lanes 15, 16, 31, 32), removal of NegC (ISWI$^{2RA/\Delta C617}$) restored relatively efficient sliding of extended g4 nucleosomes (lane 14), and moderate sliding of core g4 nucleosomes (lane 30). In keeping, removal of NegC from ISWI^{2RA} restores sliding ability with nucleosomes bearing amino acid substitutions in both H4K12 and the basic patch (Supplementary Fig. 4). Thus, with ISWI^{2RA}, NegC omission enables sliding of nucleosomes without a basic patch or extranucleosomal DNA (Fig. 3e, lanes 6, 14, 22, 30), properties normally defining SWI/SNF-family remodelers.

To examine AutoN and NegC impact *in vivo*, we expressed (in *S. cerevisiae*) mutations/truncations in Isw1 equivalent to those from our ISWI biochemistry (see Fig. 1b, 3a) and tested complementation (Supplementary Fig. 5), or dominant phenotypes (utilizing a sensitized *rsc7*Δ background, synthetically lethal with a wide range of chromatin mutants[29]). Interestingly, expression of an Isw1 derivative lacking both AutoN and NegC regulation prevented growth (Fig. 4a), consistent with rogue chromatin misregulation activity (note: loss of proper regulation by Isw1-associated proteins may also contribute).

Taken together, our work provides several conceptual advances that impact both ISWI regulation and mechanism (Fig. 4b). Regarding regulation, the prior model involved ‘positive’ regulation of ISWI by the H4 basic patch and by extranucleosomal DNA. Here, we replace that conception with an ‘inhibition of inhibition’ model, whereby these two nucleosomal epitopes function to relieve an intrinsic autoinhibition conferred by two new negative regulatory domains, AutoN and NegC. Notably, whereas AutoN functions primarily to inhibit ATPase activity, NegC functions to inhibit ATPase ‘coupling’ to DNA translocation, with structural models for NegC supporting this function. By this model, interaction of the HSS with extranucleosomal DNA does not activate the ATPase *per se*, it relieves NegC and restores ATPase coupling. Regarding mechanism, a major current model involves the HSS pushing extranucleosomal DNA into the nucleosome, providing the initial mechanical power stroke to form a translocated DNA loop on the surface of the nucleosome. However, as we observe efficient DNA translocation and nucleosome sliding following deletion of the HSS (when combined with NegC omission), the primary function of the HSS is not mechanical, but rather to regulate DNA translocation by antagonizing NegC, ensuring that coupling occurs only when extranucleosomal DNA is of sufficient length.

Methods Summary

ISWI protein derivatives were produced as N-terminal fusions to Trigger Factor and the Tet Repressor DNA-binding domain (TF-TetR-ISWI), which heterodimerizes with an unfused TetR to enable tetO binding for DNA translocation assays. Proteins were expressed in *Escherichia coli* BL21(DE3)RIL, and were purified to homogeneity as monodisperse derivatives. Chemically synthetized H4 tail peptides encompass residues K8 to D24; AutoN peptide includes ISWI residues K76 to E98.

The ATPase assays were performed at 26°C using a colorimetric assay based on the formation of a complex between inorganic phosphate and molybdate-malachite green. Reactions were an addition/omission series involving ISWI (10 pmol), pBluescript plasmid (500 ng), peptide (50 μM), DNA fragments (500 fmol), or nucleosomes (500 fmol), as indicated.

Mononucleosomes utilized purified recombinant *Drosophila* histones expressed in *E. coli* BL21(DE3)RIL, assembled with 147bp or 200bp DNA fragments (isolated from a plasmid by restriction digests, and containing the 601 strong positioning sequence) by a saltdialysis linear gradient. For the 200bp fragment, the 601 sequence was centrally located.

The DNA translocation assays measured plasmid supercoils generated by a single ISWI protein anchored via its TetR fusion to a previously relaxed (by *E. coli* TopoisomeraseI) plasmid DNA containing a single tetO operator sequence (Supplementary Fig. 2). Deproteinized samples were loaded on a 1.3% agarose gel, subsequently stained in ethidium bromide and scanned on a Typhoon Trio (Amersham, GE).

The nucleosome sliding assays were performed at 26°C using a 1:2 enzyme:substrate molar ratio, except in the titration series. The reactions were stopped by adding competitor pBluescript plasmid DNA, and loaded on a 4.5% (37.5:1) native polyacrylamide gel, subsequently stained in ethidium bromide and scanned on a Typhoon Trio (Amersham, GE).

Full Methods

Reagents

ISWI protein derivatives were expressed in *Escherichia coli* BL21(DE3)RIL, upon 0.5mM IPTG induction at OD_{600}~0.5 during 24hours at 15°C, as complexes from 2 vectors: one – pCDFDuet1- bears a TetR-$(His)_7$ gene, the other - pCold TF (Takara) lacking the His-tag - a gene fusion containing Trigger Factor-3C cleavage site-TetR-ISWI-Flag. Between TetR and ISWI was inserted an eight amino-acid sequence GGQGGQGG. The properly assembled complex contains a heterodimer of TetR obtained by two successive affinity purifications. The bacteria cell extracts were first mixed with Ni-NTA agarose resin (Qiagen), capturing the complex and the unwanted TetR homodimer. The sample eluted from the resin was then purified using the Flag tag and anti-Flag M2 affinity gel and eluted with 3×FLAG peptide (Sigma) to obtain the desired complex. Then, Trigger Factor was cleaved from the complex using HRV 3C protease (Novagen) using 1 Unit of protease / 0.1 mg of complex by incubating 16 hours at 4°C, in presence of 10mM β-mercaptoethanol and 0.5mM EDTA.

Finally, the purification of the complex was achieved by a gel filtration step on two S200GL 10/300 (Amersham, GE) in series. Obtained complexes were homogen and monodisperse.

Chemically synthetized H4 tail peptides encompass residues K8 to D24; AutoN peptide includes ISWI residues K76 to E98.

Mononucleosomes were produced from single recombinant *Drosophila* histones expressed in *Escherichia coli* BL21(DE3)RIL, purified as inclusion bodies, and assembled in octamers by salt-dialysis, essentially as described[31]. The 200 and 147 bp DNA fragments containing the 601 positioning sequence[32] were respectively produced from plasmids pUC12×601 digested by AvaI, and pST55-16×NCP601a digested by EcoRV. For the 200bp fragment, the 601 sequence was centrally located. The DNA fragments were purified from the backbone using preparative electrophoresis (PrepCell, BioRad) 4.5% (37.5:1) native polyacrylamide gel running at 400V constant in 0.5×TBE, with TE as elution buffer. Mononucleosome assemblies were performed by titration reactions, as 50µl reaction containing 40pmol of DNA fragment in 2M KCl mixed with a variable amount of histone octamers covering the equimolar ratio, with a linear salt-gradient dialysis applied from 2M to 50mM KCl, essentially as described[31], using an Econo-Pump (BioRad) and Slide-A-Lyser Mini Dialysis Units 7000MWCO (ThermoScientific).

ATPase Assay

Measurement of ATP hydrolysis was based on the formation of a complex between inorganic phosphate and molybdate-malachite green. ATPase assays were performed in 10mM Hepes pH=7.3, 20mM KOAc, 5mM $MgCl_2$, 0.5mM DTT, 0.1 mg/ml BSA and 5% glycerol, in presence of 1mM ATP. After 30min incubation at 26°C with 500rpm shaking in a Thermomixer (Eppendorf), 800µl of MGAM reagent (3 volumes of MG=0.045%(w/v) of malachite green in 0.1N HCl, mixed with 1 volume of AM=4.2%(w/v) of ammonium molybdate in 4N HCl) were added, followed one minute later by 100µl of 34% (w/v) Na_3Citrate. Measures were performed at OD=650nm 10 minutes later. In the plasmid DNA and peptides experiments, 10pmol of ISWI were used with or without 500ng of pBluescript plasmid and/or 50µM of peptide. In the mononucleosomes or DNA fragments experiments, 10pmol of ISWI were in presence of 500 fmol of nucleosomes or DNA fragments respectively.

DNA Translocation Assay

The 20µl reaction was containing 10pmol of ISWI, with or without 15µM of peptide, in presence of 250ng of previously relaxed plasmid, 1mM ATP, 2.5U of TopoisomeraseI (NEB) in NEB1 1× buffer, 1 mg/ml BSA. Experiments were performed at 30°C for 2 hours (or shorter during time course), followed by heat inactivation at 65°C for 20 min. Deproteinization was performed by adding 2µl of Proteinase K at 10mg/ml and 1µl of SDS 20% and incubated at 50°C for 1h. Reactions were subsequently precipitated in ethanol, prior loading on a 1.3% agarose gel run for 3h at 130V. Gels were stained 20 minutes in a 1µg/ml ethidium bromide solution, and scanned on a Typhoon Trio (Amersham, GE). Time course experiments are performed using a starting reaction corresponding to a scale-up of amounts above, proportional to the number of desired aliquots.

Sliding Assay

100 fmol (or variable for titration) of ISWI were incubated in 10mM Tris pH=7.4, 50mM KCl, 3mM $MgCl_2$, 0.1 mg/ml BSA, 1mM ATP, in presence of 200 fmol of mononucleosomes for 90 min at 26°C with shaking at 500rpm in a thermomixer (Eppendorf). 10μl reactions were stopped by adding 200ng of competitor DNA (pBluescript plasmid) and incubate additional 30 min as previously. Reactions were loaded using glycerol 10% on a 4.5% (37.5:1) native polyacrylamide gel and run in 0.4× TBE for 55 minutes at 110V constant. Gel was stained 10 minutes in a 1μg/ml ethidium bromide solution, and scanned on a Typhoon Trio (Amersham, GE). Time course experiments are performed using a starting reaction corresponding to a scale-up of amounts above, proportional to the number of desired aliquots.

In Vivo Yeast Experiments

Plasmids expressing Isw1 derivatives were generated by marker conversion to *Leu2* of pRS416-Isw1-WT[33], followed by site-directed mutagenesis (via PCR) generating AAA mutations and ΔC truncations. Final constructs were verified by sequencing.

Complementation Assay—The growth of *Saccharomyces cerevisiae* strain TOH1358 lacking *CHD1* and *ISW1* genes is temperature sensitive[34]. Complementation experiments were performed by transforming the strain with plasmids expressing various derivatives of Isw1 under control of the endogenous promoter, performing tenfold spot dilutions, and observing the rescue of the growth ability at high temperature. Plate medium is synthetic complete lacking leucine.

Testing the Dominant Negative Phenotype—Here, we used a sensitized genetic background strain YBC 2233, lacking *isw1* and *rsc7*. The strain was transformed with plasmids expressing separate Isw1 derivatives under control of the endogenous ISW1 promoter, selecting for colonies on synthetic complete growth medium lacking leucine. Growth ability (in spot dilution format) was tested at 30°C on SC-Leu plates lacking leucine, that either lacked or contained 5-FOA (which enforces the loss of a *URA3*-marked plasmid bearing *RSC7*).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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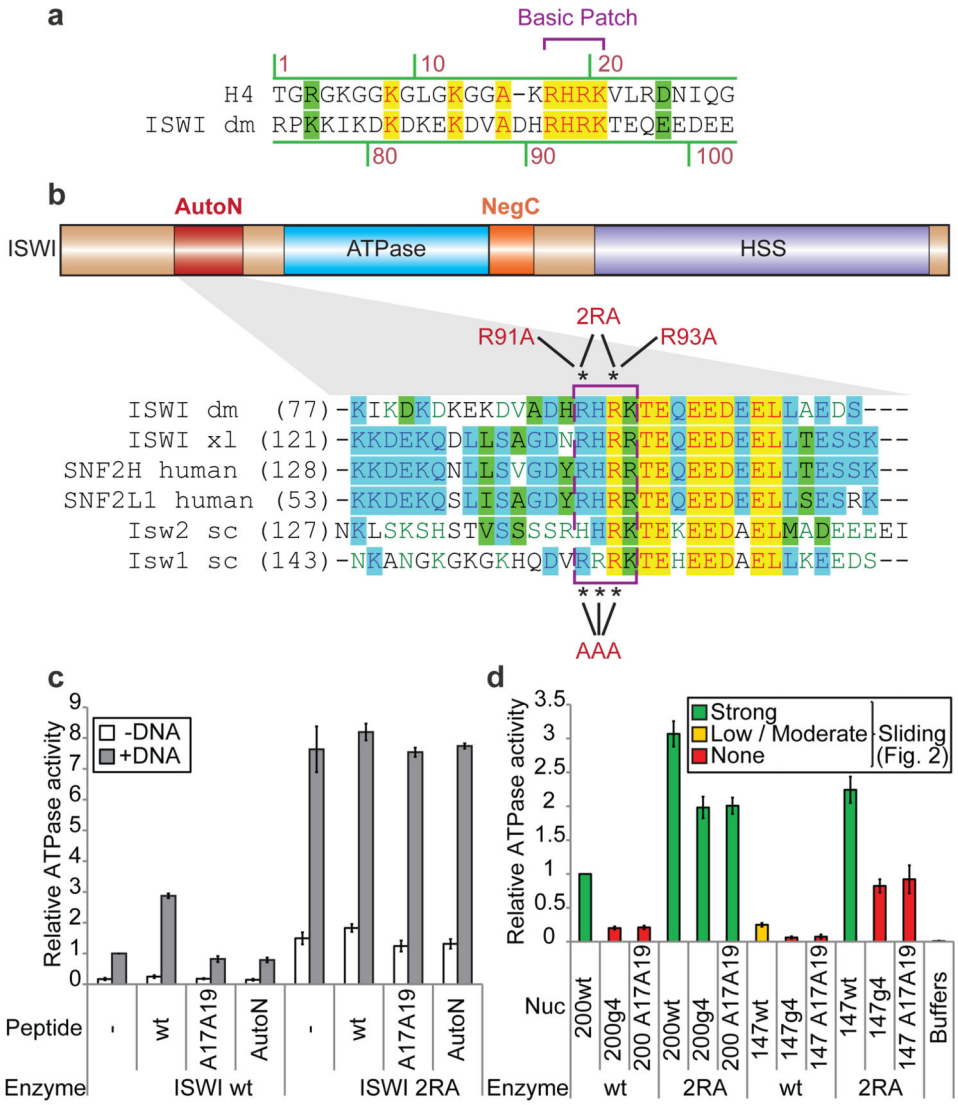

Figure 1. AutoN resembles the histone H4 tail basic patch and restricts ISWI ATPase activity

a, Alignment of ISWI (*Drosophila*) and the histone H4 N-terminus, with the basic patch depicted. **b**, ISWI protein and domains/regions, including ATPase, HAND-SANT-SLIDE (HSS), and new functional regions (AutoN and NegC), with zoom of AutoN alignments for ISWI orthologs: *Drosophila* (dm), *Xenopus* (xl), human, and *Saccharomyces cerevisiae* (sc). **c**, Mutation of AutoN (2RA) hyperactivates ISWI ATPase activity and bypasses H4 tail stimulation by peptides. Values, mean of ≥3 experiments, normalized to ISWI^{wt} with DNA. Error bars +/− s.e.m. **d**, AutoN (2RA) mutation stimulates ISWI ATPase activity with mononucleosomes: native (wt), H4 tailless/globular (g4), or basic patch mutations (A17A19). Color code (inset) reflects nucleosome sliding activity (in Figure 2c,d). Values, mean of ≥3 experiments, normalized to ISWI^{wt}. Error bars +/− s.e.m.

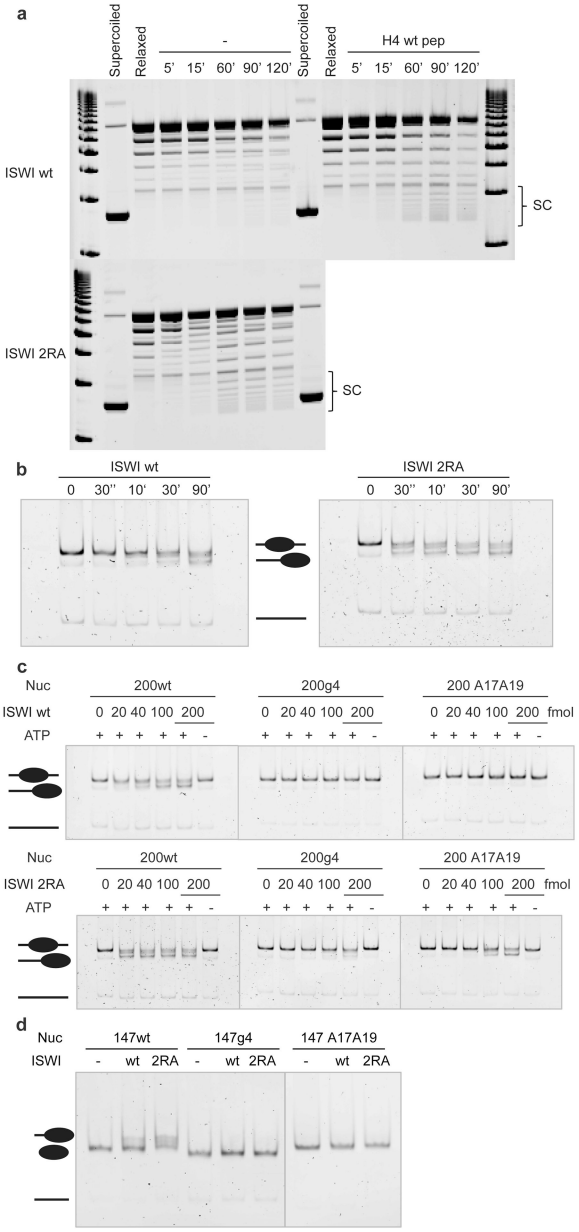

Figure 2. AutoN mutation (ISWI^{2RA}) increases DNA translocation and nucleosome sliding

a, Comparative DNA translocation activity (see Supplementary Fig. 2) of ISWI derivatives. Translocation generates supercoiled topoisomers (SC). **b**, Comparative sliding of extended nucleosomes by ISWI derivatives, as a time course, and **c**, their reliance on H4 tail epitopes, in titration series. **d**, Comparative sliding activity on core nucleosomes reveals a reliance on H4 tail epitopes. For b and d, enzyme:substrate molar ratio is 1:2.

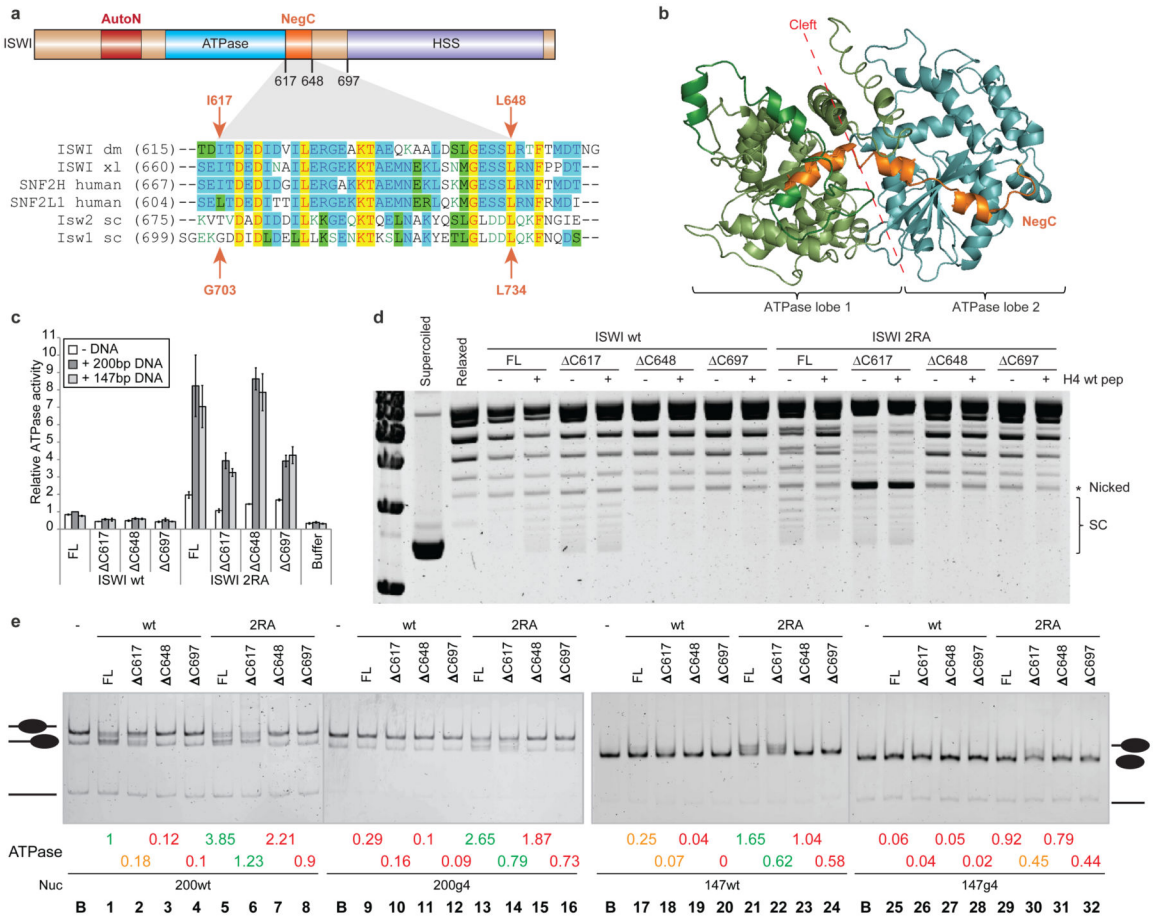

Figure 3. NegC inhibits the coupling of ATP hydrolysis to DNA translocation, and nucleosome sliding

a, NegC conservation in ISWI orthologs. ISWI protein, with zoomed alignment of NegC from ISWI orthologs: *Drosophila* (dm), *Xenopus* (xl), human, and *Saccharomyces cerevisiae* (sc). **b,** Modelisation of ISWI$^{\Delta C697}$ showing NegC (orange) traversing the cleft (red dash line) from the ATPase lobe #2 (blue) to ATPase lobe #1 (green). Model generated using Phyre[2]; depicted with PyMol[30]. **c,** The AutoN/2RA mutation elicits robust DNA-dependent ATPase activity independent of the HSS. Values, mean of three experiments, normalized to ISWI^{wt}, with 200bp DNA fragment. Error bars +/− s.e.m. **d,** Deletion of NegC via truncation restores DNA translocation. **e,** Impact of NegC region on sliding activities of core and extended nucleosomes (and H4 tailless versions). ATPase activities (values, colored by impact on sliding: high (green), moderate/low (orange), absent (red)). Enzyme:substrate molar ratio 1:2 . B (Buffer).

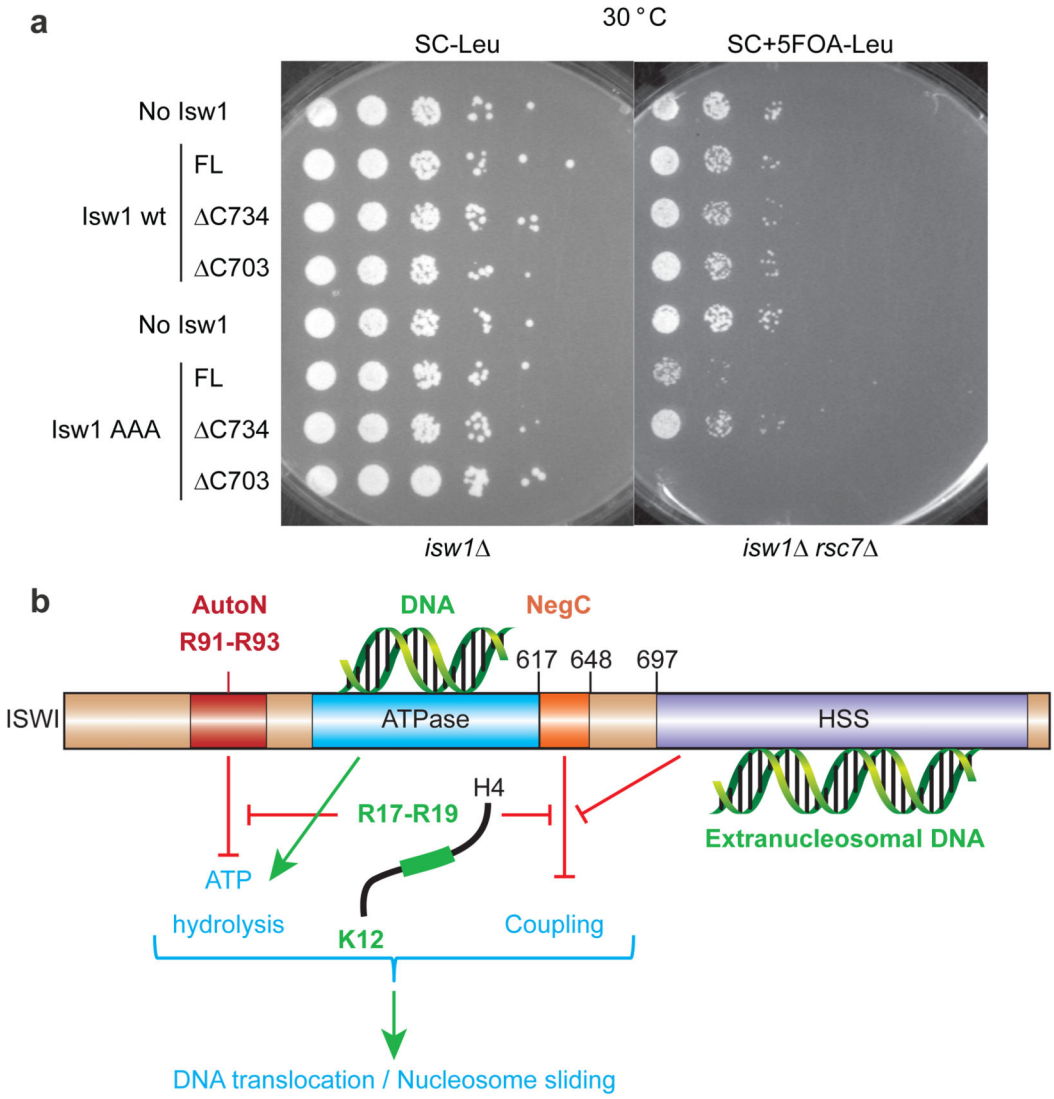

Figure 4. Expression of ISWI derivatives *in vivo*, and regulation model for ISWI

a, Isw1 lacking AutoN and NegC regulation greatly impairs growth of an *S. cerevisiae* strain sensitized for chromatin misregulation (*rsc7*Δ). Isw1 derivatives expressed in an *isw1*Δ *rsc7*Δ [p *RSC7*$^{+}$.URA3] strain. 5-FOA enforces the loss of the *URA3*-marked *RSC7*$^{+}$ plasmid, imposing a *rsc7*Δ genotype. **b**, Logic of ISWI ATPase regulation. The ISWI ATPase activity is positively regulated by DNA in the ATPase cleft. ISWI is also negatively regulated by two intrinsic domains: AutoN and NegC. AutoN inhibits the ATP hydrolysis rate, and is relieved by the basic patch (R17-R19) of the H4 tail, whereas NegC inhibits ATPase coupling to DNA translocation, and is relieved by the HSS domain binding to sufficient extranucleosomal DNA: defining the ‘inhibition of inhibition’ mode of ISWI regulation. (Note: an additional lysine (H4K12) also promotes coupling.)