Releasing the brakes on a chromatin-remodeling enzyme

Benjamin J Manning & Craig L Peterson

Chromatin-remodeling enzymes use the energy from ATP hydrolysis to mobilize, disrupt or change the histone composition of nucleosomes, facilitating nearly every nuclear event. Two recent studies indicate that remodeling enzymes harness the power of an ancient constitutively active DNA translocase and that different remodeling enzymes may use specialized coupling domains that communicate the presence of nucleosomal epitopes to regulate translocase and remodeling activity.

During interphase of the eukaryotic cell cycle, the bulk of genomic DNA is packaged into folded nucleosomal arrays that impede all DNA-mediated events. These events nevertheless function efficiently, owing in large part to the action of a plethora of ATP-dependent chromatin-remodeling enzymes. These enzymes typically function as large multisubunit complexes, all of which harbor a catalytic ATPase subunit that is related to the SF2 superfamily of DNA helicases[1]. The different catalytic subunits of four well-characterized remodeling complexes define four distinct ATPase subfamilies that contain, in addition to their core ATPase-homology domain, characteristic N- or C-terminal sequence motifs (ISWI, Chd1, Snf2 and Swr1; **Fig. 1**)[2]. Two recent studies, one published in *Nature* by Clapier *et al.*[3] and the other in *Nature Structural & Molecular Biology* by Mueller-Planitz *et al.*[4], provide intriguing evidence that the minimal conserved ATPase domain is sufficient to bind and remodel nucleosomes, and that adjacent inhibitory modules regulate this core ATPase by blocking its action unless appropriate nucleosomal epitopes are engaged by the enzyme.

Over the past few years, single-molecule and ensemble studies have shown that chromatin-remodeling enzymes employ ATP-dependent DNA-translocase activity[1], consistent with the helicase homology of ATPase subunits. Such data have led to a broadly accepted model for ATP-dependent remodeling in which the catalytic ATPase or DNA translocase subunit 'pumps' DNA around the histone octamer and leads to nucleosome mobilization[1]. Although it seems likely that DNA translocation lies at the heart of all ATP-dependent remodeling reactions, it is still unclear how different remodeling enzymes switch this translocase activity on or off, or how they use this activity to direct formation of distinct remodeling products.

The ISWI subfamily of remodeling enzymes requires the histone H4 N-terminal tail to mobilize nucleosomes in *cis*[5,6], and its activity is regulated by histone H4 Lys16 acetylation[7]. Functional interaction with the H4 tail is consistent with the observation that several enzymes, including members of the ISWI subfamily, bind and initiate the remodeling reaction on the nucleosomal surface where the H4 tail protrudes[8,9]. However, enzymes that harbor Snf2-like ATPases (for example, SWI/SNF) also bind to this location, but their activity does not require histone tails. How the H4 tail specifically interacts with and regulates ISWI enzymes has long been a mystery.

In their paper, Clapier *et al.*[3] identify an N-terminal domain, called AutoN, which is located within the N terminus of ISWI ATPases and contains striking homology to a patch of basic residues within the H4 tail. Intriguingly, a two-amino-acid substitution (R91A, R93A or both (2RA)) within AutoN creates an enzyme that no longer requires the H4 tail for high levels of ATPase activity, DNA translocation or remodeling of mononucleosome substrates. Indeed, the 2RA substitution even allows ISWI to more effectively use free DNA as a cofactor for ATPase activity. These data indicate that AutoN functions as a 'brake' on the ISWI ATPase domain, preventing the use of free DNA and coupling ATPase and translocase activity to the presence of the H4 tail (**Fig. 2a**,**b**).

Although the H4 homology within AutoN is not found in other families of remodeling ATPases, it is located directly adjacent to a

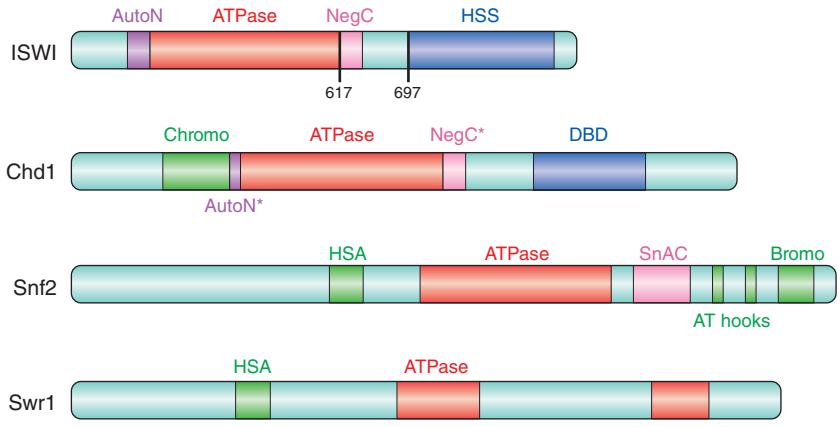

Figure 1 Four distinct families of chromatin-remodeling ATPases. Asterisks indicate domains in Chd1 similar in sequence to those in ISWI.

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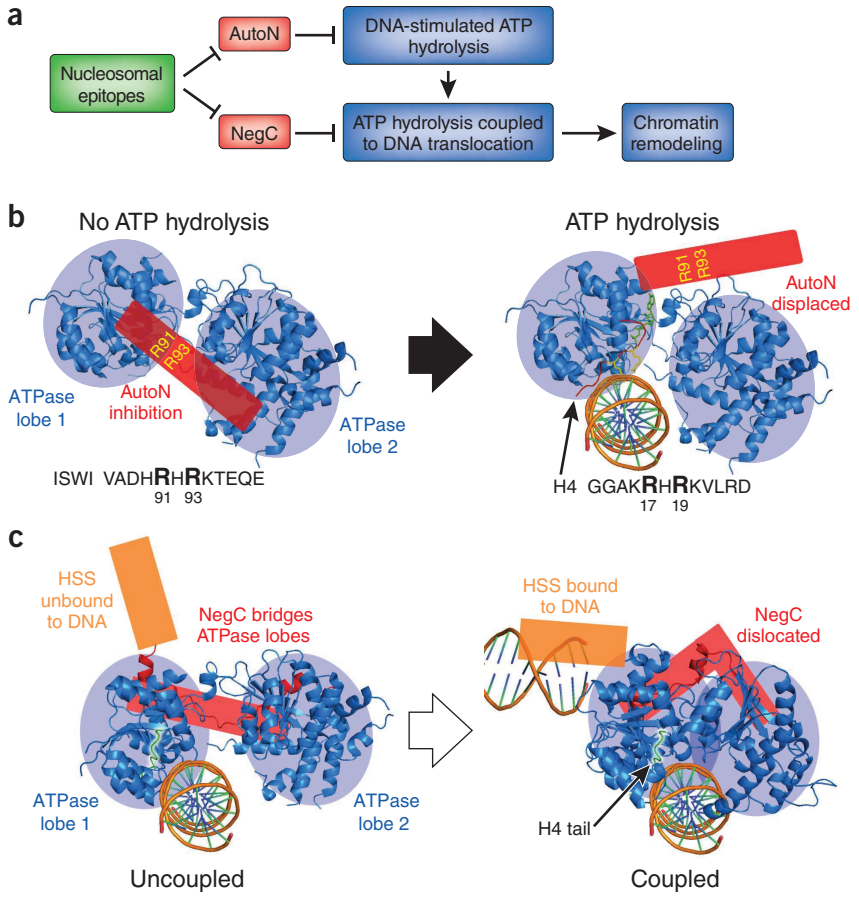

Figure 2 Model for regulation of ISWI ATPase activity and DNA translocation. (**a**) Flowchart of the proposed ISWI remodeling reaction and regulatory inputs. (**b**) The ISWI AutoN domain (red) negatively regulates ATP hydrolysis by contacting the ATPase lobes (blue, left). ATPase activation may involve displacement of AutoN by the H4 tail and DNA (right). (**c**) NegC negatively regulates the coupling of ATP hydrolysis and DNA translocation by forming a bridge (red) between ATPase lobes (blue). Interaction of the C-terminal DNA-binding domain (HSS, orange) with DNA disrupts the NegC bridge, thereby activating coupling. This coupling step also appears to require the H4 tail. Models were generated with PyMOL (http://www.pymol.org/), using the crystal structures of the Chd1 ATPase[10] and DNA-binding domain bound to DNA[23].

stretch of acidic amino acids that is also found within the N terminus of the Chd1 remodeling enzyme[10] (**Fig. 1**). Like ISWI, the ATPase activity of Chd1 is preferentially activated by nucleosomes, and its remodeling activity is most active with nucleosomes that contain the H4 tail. Remarkably, the crystal structure of the Chd1 ATPase shows that the N-terminal acidic patch (called the 'chromo wedge') contacts the second ATPase lobe and blocks the ability of DNA to bind and activate ATPase activity[10]. Indeed, alterations of this Chd1 acidic patch or removal of the entire N terminus creates a Chd1 ATPase that cannot distinguish DNA from nucleosomes and is thus equally activated by both. Furthermore, alterations in the acidic patch enhance the ability of Chd1 to remodel nucleosomes that lack the H4 tail, similar to the effect of the 2RA substitution within ISWI[10]. Taken together, these studies suggest that an AutoN-like region within ISWI and Chd1 ATPases may be an allosteric regulator of ATPase activity, generally functioning to prevent use of DNA as a cofactor for ATPase activation. This inhibitory domain must eventually be dislodged from the ATPase lobes by an unknown mechanism that couples both DNA and the H4 tail. Given that the activity of ISWI enzymes is more strictly H4 tail–dependent than that of Chd1, one might speculate that the additional H4 homology within the ISWI AutoN domain increases specificity for this nucleosomal epitope.

Although disruption of the ISWI AutoN domain restores high ATPase activity in the absence of the H4 tail, wild-type levels of nucleosome mobilization still require this nucleosomal epitope[3]. This indicates an additional role for the H4 tail to couple ATP hydrolysis to DNA translocation. This coupling function is also dependent on free DNA adjacent to the nucleosome, a hallmark of both ISWI- and Chd1-like enzymes[11–13]. Combining the disruption of AutoN with removal of the C-terminal DNA-binding domain of ISWI (the HSS domain) creates an enzyme with very high ATPase activity that lacks DNA-translocase activity[3]. Intriguingly, removal of the HSS domain also cripples nucleosome mobilization even in the presence of the H4 tail or linker DNA, though activity is observed when 10- to 30-fold higher enzyme concentrations are used, as demonstrated by Mueller-Planitz *et al.*[4]. Thus, the HSS domain is required to effectively integrate both of these nucleosomal inputs and to couple them to ATP hydrolysis and the mechanical work of chromatin remodeling.

How does the HSS domain perform this coupling role? Taking a lesson from their work on AutoN, Clapier *et al.*[3] find that DNA-translocase activity, coupling and nucleosome remodeling are restored in the absence of the HSS domain as long as the region immediately C terminal to the ISWI ATPase domain is absent (ISWI-C617, **Fig. 1**). Additional deletions define a new inhibitory domain, called NegC, that is located between the ATPase core and the HSS DNA-binding domain. Interestingly, the NegC domain was previously noted in the Chd1 crystal structure as a domain, conserved within ISWI and Chd1 family members, that docks against the two ATPase lobes and that may lock Chd1 in an inactive conformation[10]. These data support a general model in which a NegC domain locks the ATPase lobes in a conformation that uncouples ATPase activity from DNA translocation, and releasing this NegC brake appears to require a C-terminal DNA-binding domain. Given that both the Chd1 and ISWI DNA-binding domains harbor SANT domains[14,15], which are potential histone tail–interaction modules[16], these domains may directly integrate signals from both linker DNA and the H4 tail (**Fig. 2a**,**c**). However, Mueller-Planitz *et al.*[4] show that an ISWI derivative that lacks the HSS domain still responds to the H4 tail, so there are clearly other domains within ISWI that recognize this nucleosomal epitope.

The identification of inhibitory roles for the AutoN and NegC domains implies that the chromatin-remodeling activities of ISWI and Chd1 enzymes are regulated by dramatic conformational changes, triggered by nucleosomal epitopes, that derepress an inherently active DNA-stimulated translocase domain. Such conformational changes are supported by detailed ATPase kinetic studies reported by Mueller-Planitz *et al.*[4], indicating that ISWI is present in two distinct enzyme conformations in the absence of DNA. Addition of DNA causes dramatic protease hypersensitivity within ISWI, and remarkably, the sites of cleavage map to residues adjacent to NegC and at the key arginine residues within AutoN[4]. These

results suggest that nucleosome interactions stabilize a conformation of ISWI that is permissive for DNA translocation, and on the basis of identification of AutoN and NegC, this conformational change probably involves removal of both of these ATPase brakes. Furthermore, in the absence of AutoN and NegC, ISWI can even mobilize nucleosome core particles that lack both linker DNA and the H4 tail, which suggests that it now represents a 'bare bones' remodeling enzyme that has lost all regulatory hallmarks of an ISWI-like enzyme[3].

These studies with ISWI and Chd1 suggest that different types of remodeling enzymes exploit and then uniquely regulate the activity of a minimal DNA translocase or nucleosome-mobilization module. For Chd1 and ISWI, which share many properties, this involves the use of AutoN and NegC domains that prevent translocase activity with naked DNA, thus coupling activity to nucleosomal substrates and the H4 tail. But what about other remodeling enzymes, such as Swr1-like (for example, SWR-C or INO80) or Snf2-like enzymes (for example, yeast or human SWI/SNF or BAF) (**Fig. 1**)? Swr1-like enzymes appear to be dependent on other subunits even for ATPase activity[17], and SWR-C is specialized for histone H2A.Z deposition and cannot even mobilize nucleosomes[18]. Furthermore, Snf2-like enzymes do not require histone tails for activity, and their ATPase activity is fully activated by DNA alone[1]. Interestingly, Snf2 homologs contain a C-terminal AT hook DNA-binding domain, and genetic studies suggest that it may be functionally important[19]. In addition, Sen *et al.*[20,21] have identified a domain, called SnAC, that is essential for coupling ATP hydrolysis to chromatin remodeling. Interestingly, SnAC lies between the ATPase domain and this C-terminal DNA-binding domain. Moreover, SnAC has limited sequence similarity to residues positioned adjacent to NegC of ISWI and implicated in coupling activity for Chd1 (discussed in ref. 22). Although Snf2-like ATPases lack a recognizable NegC domain, they may contain a domain of similar function, or other subunits may fulfill such a role. Thus, it seems likely that these enzymes will also employ accessory domains that counteract inhibitory influences exerted on the ATPase domain, directing distinct remodeling outcomes.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Sibling rivalry: competition between MHC class II family members inhibits immunity

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Peptide loading of major histocompatibility complex (MHC) class II molecules in the endosomes and lysosomes of antigen-presenting cells is catalyzed by human leukocyte antigen-DM (HLA-DM) and modulated by HLA-DO. In a structural study in this issue, Guce *et al.* show that HLA-DO is an MHC class II mimic and functions as a competitive and essentially irreversible inhibitor of HLA-DM activity, thereby inhibiting MHC class II antigen presentation.

Antigen presentation, the recognition of MHC class II (MHCII) molecules that display pathogen-derived peptide fragments by $CD4^+$ T cells, is essential for the initiation of adaptive immune responses. MHCII molecules bind peptides in the endocytic pathway of antigen-presenting cells (APCs) by a process directly catalyzed by their interaction with a non–peptide binding MHCII homolog, in humans called HLA-DM[1,2]. HLA-DM activity is modulated in the endosomes of APCs by its association with another MHCII-like protein called HLA-DO. Remarkably, initial biochemical studies demonstrated that these two MHC-encoded proteins have directly opposing roles: HLA-DM facilitates MHCII peptide binding, whereas HLA-DO inhibits it by associating with and somehow disabling HLA-DM[3,4]. Consistent with these observations, overexpression of HLA-DO in cell lines and transgenic mice for the most part results in reduced antigen presentation directly correlating with decreased peptide binding[3–5]. However, when cell line–based or *ex vivo* antigen-presentation assays were used to define the impact of HLA-DO expression on the presentation of specific antigenic peptides, the role of HLA-DO as an HLA-DM inhibitor was less clear. Studies have been published showing that in different situations, HLA-DO inhibits, promotes or has no effect

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