OPINION

The SANT domain: a unique histone-tail-binding module?

Laurie A. Boyer, Robert R. Latek and Craig L. Peterson

Chromatin-remodelling complexes have an important role in all DNA-mediated processes and, although much is known about how these enzymes regulate chromosomal DNA accessibility, how they interact with their histone substrates has remained unclear. However, recent studies have indicated that the SANT domain has a central role in chromatin remodelling by functioning as a unique histone-interaction module that couples histone binding to enzyme catalysis.

Eukaryotic DNA is organized in the nucleus as compact, 30–400-nm chromatin fibres by its association with both histone and non-histone proteins. This complex structure is generally inaccessible to DNA-binding proteins, so the manipulation of chromatin structure is probably a prerequisite for all DNA transactions. The nucleosome is the most basic level of chromatin organization and consists of an octamer of core histones (two copies each of H2A, H2B, H3 and H4), around which 1.75 superhelical turns of DNA is wrapped[1]. Each of the core histones contains a central, structured domain that is sufficient for mediating histone–histone interactions as well as DNA organization. In addition, all four core histones contain highly basic amino-terminal 'tail' domains (histone H2A also has a carboxy-terminal tail) that extend from the surface of the nucleosome[2]. The histone tails are essential for the higher-order folding of chromatin fibres, and they also provide binding sites for non-histone regulatory proteins[3]. Both of these functions can be regulated by the post-translational modification of residues in the histone tails (for example, acetylation, methylation and phosphorylation), and the tails are therefore key targets for the regulation of chromatin-fibre structure and function[4,5].

Two classes of highly conserved enzymes have been implicated as regulators of the accessibility of chromatin-packaged DNA[6,7] (BOX 1). The ATP-dependent class of chromatin-remodelling enzymes uses the energy derived from ATP hydrolysis to disrupt histone–DNA interactions, whereas the second class includes enzymes that catalyse the covalent attachment or removal of post-translational modifications (for example, lysine acetylation, serine phosphorylation, lysine and arginine methylation, ubiquitylation and ADP ribosylation). Although these two classes of enzyme are functionally distinct, several protein motifs are commonly found among the subunits of both types of remodelling enzyme (for example, bromodomains, chromodomains and SANT domains (for 'switching-defective protein 3 (Swi3), adaptor 2 (Ada2), nuclear receptor co-repressor (N-CoR), transcription factor (TF)IIIB')). In some cases, bromodomains allow remodelling complexes to selectively interact with histone tails that contain acetylated lysine residues[8–10], and a subset of chromodomains might carry out an analogous function to allow the recognition of methylated lysine residues[11,12]. Interestingly, the SANT domain is even more broadly represented among chromatin-remodelling enzymes than bromodomains or chromodomains, which implies that it has an essential role in regulating chromatin accessibility.

The SANT domain

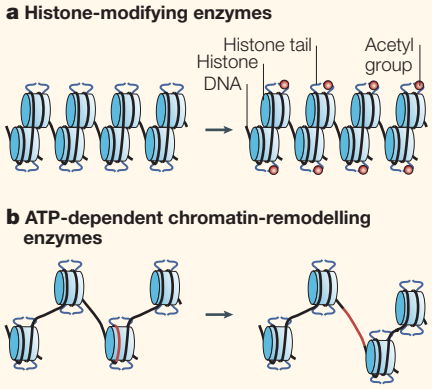
The SANT domain was initially identified as an approximately 50-amino-acid motif that is present in nuclear receptor co-repressors, and it was subsequently found in the subunits of many chromatin-remodelling complexes[13] (FIG. 1a; for further sequence alignments and a phylogenetic analysis, see SANT supplementary information in Online links). Further sequence analysis highlighted a strong similarity to the DNA-binding domain (DBD) of Myb-related proteins[13]. Myb DBDs contain tandem repeats of three α-helices that are arranged in a helix–turn–helix motif [14,15] (FIG. 1b). Each α-helix contains a highly conserved, bulky aromatic residue, and collectively these residues form a hydrophobic core that is essential for the overall structural fold[14,15]. Initial sequence alignments, as well as secondary structure predictions, indicated that the SANT domain also consists of three α-helices, each of which contains a corresponding, bulky aromatic residue[13] (FIG. 1a). A recent high-resolution X-ray structure of a SANT domain confirmed the overall structural similarity to the Myb DBD[16].

The Myb–DNA co-crystal structure indicates that residues in the third — or 'recognition' — α-helix make sequence-specific contacts with DNA, and several basic residues that immediately follow the third helix are in a position to contact the phosphate groups of DNA[14]. Whereas, in many cases, SANT domains do contain several basic residues on the carboxy-terminal side of the third α-helix, several key residues that contact DNA in the Myb DBD are not conserved in SANT domains. In addition, SANT domains contain consecutive hydrophobic residues in the putative recognition helix that are predicted to be incompatible with DNA binding (FIG. 1a). Furthermore, in contrast to the basic character of the Myb DBD, SANT domains are

Box 1 | **Chromatin-remodelling complexes**

Two classes of highly conserved enzymes have been implicated as regulators of the accessibility of DNA in chromatin:

Histone-modifying enzymes. This group of enzymes adds or removes post-translational modifications to amino-terminal histone tails (see figure part **a**, in which histone acetylation is shown). Modifications to the tails function to decondense chromatin and/or recruit other enzymes or proteins to the nucleosomes. The most well-understood enzymes in this class are the histone acetyltransferases (HATs) and the histone deacetylases (HDACs), which alter the acetylation state of specific lysines in the amino-terminal tails of histones H3 and H4.



ATP-dependent chromatin-remodelling enzymes. This class of enzymes is related to the megadalton yeast SWI/SNF complex and can use the free energy from ATP hydrolysis to disrupt DNA–histone interactions (see figure part **b**), which leads to the mobilization of nucleosomes within the chromatin fibre.

Shown in the table below are a subset of chromatin-remodelling complexes, including those that are the primary focus of this article and contain SANT domains.

Enzyme type	Complex	Organism	Function*
Histone-modifying enzymes	SAGA	Yeast	HAT
	PCAF	Humans	HAT
	STAGA	Humans	HAT
	NuA4	Yeast	HAT
	HDAC1	Humans	HDAC
	SMRT/N-CoR	Humans	HDAC
	RPD3	Yeast	HDAC
	SUV39H1	Humans	HMT
	SET1	Yeast	HMT
ATP-dependent chromatin -remodelling enzymes	RSC	Yeast	
	SWI/SNF	Flies, humans and yeast	
	NuRF	Flies and humans	
	CHRAC	Flies and humans	
	ACF	Flies	
	ISW1/2	Yeast	
	RSF	Humans	
	Mi-2/NuRD	Humans	

* Function is only specified for the histone-modifying enzymes. ACF, ATP-utilizing chromatin assembly and remodelling factor; CHRAC, chromatin-accessibility complex; HDAC1, histone-deacetylase protein 1; HMT, histone methyltransferase; ISW1/2, imitation-switch protein 1/2 (yeast homologues of *Drosophila melanogaster* Iswi); Mi-2/NuRD, nucleosome-remodelling complex; NuA4, nucleosome acetyltransferase of histone H4; NuRF, nucleosome-remodelling factor; PCAF, p300/CBP-associated factor; RSC, remodel the structure of chromatin; RSF, remodelling and spacing factor; SAGA, Spt–Ada–Gcn5-acetyltransferase; SET1, SET-domain-containing protein 1; SMRT/N-CoR, silencing mediator of retinoid and thyroid receptors/nuclear receptor co-repressor; STAGA, SPT3–TAF(II)31–GCN5L acetylase; SUV39H1, suppressor of variegation 3–9 homologue 1; SWI/SNF, switching-defective/sucrose non-fermenting.

characterized by a relatively acidic isoelectric point (pI) and a negative electrostatic surface (FIG. 2). Therefore, the SANT domain seems to be functionally divergent from the canonical Myb DBD.

SANT and chromatin remodelling

Genetic studies in yeast have investigated the importance of SANT domains in the subunits of several chromatin-remodelling complexes — in Swi3 of SWI/SNF (switching-defective/sucrose non-fermenting), in Rsc8 of RSC (remodel the structure of chromatin) and also in Ada2. SWI/SNF and RSC are both ATP-dependent remodelling complexes and Ada2 is a subunit of numerous Gcn5 (general control of amino-acid-synthesis protein 5)-containing histone-acetyltransferase (HAT) complexes. Remarkably, small deletions in each of these SANT domains result in transcriptional defects that are very similar to those that occur after the complete deletion of the genes for these subunits[17–19]. By contrast, removal of the bromodomain from the ATP-dependent remodelling enzymes yeast Swi2/Snf2 or *Drosophila melanogaster* brahma, or from the Gcn5 HAT, produces only a mild[20–22] or unaltered phenotype *in vivo*[23]. Although the essential role that SANT domains have in SWI/SNF-like complexes remains enigmatic[18], several recent reports, which are outlined below, indicate that SANT domains might couple histone-tail binding to enzymatic activity.

SANT and histone acetylation. An essential role for the SANT domain in histone acetylation was highlighted by the analysis of Ada2, a conserved subunit of numerous Gcn5-containing HAT complexes (for example, yeast ADA (Ada acetyl transferase) and SAGA (Spt–Ada–Gcn5-acetyltransferase)). Whereas wild-type complexes that contain Gcn5 and Ada2 have robust HAT activity towards nucleosomal substrates, complexes that contain an Ada2 subunit that has a small deletion in the SANT domain are inactive in nucleosomal HAT assays[18,19]. In addition, HAT complexes that contain a deletion in the Ada2 SANT domain show a reduced ability to bind to unacetylated histone-H3 tails[18].

More detailed kinetic analyses using a dimeric Gcn5–Ada2 complex showed that Ada2 enhances the catalytic efficiency of the Gcn5 HAT on an H3 amino-terminal peptide, core histones and nucleosomal histone substrates by 10-fold, 31-fold and 34-fold, respectively[18]. Remarkably, these stimulatory effects are eliminated by a small deletion in the SANT domain. Furthermore, the SANT-dependent enhancement of HAT activity involves both an enhanced affinity for the histone substrate (decreased K_m) and an increase in the rate of enzyme catalysis (k_{cat}). Collectively, these data strongly indicate an essential role for the Ada2 SANT domain in regulating the HAT activity of the Gcn5 catalytic subunit.

SANT and histone deacetylation. The nuclear receptor co-repressors SMRT (silencing mediator of retinoid and thyroid receptors) and N-CoR are subunits of histone deacetylase (HDAC) complexes, and they recruit deacetylase activity to target genes through interactions with unliganded nuclear receptors. N-CoR and SMRT both contain a pair of closely spaced SANT domains (SANT1 and SANT2). In each protein, SANT1 and SANT2 share only 30% identity with each other, and recent work has highlighted distinct roles for each SANT domain in the function of SMRT/N-CoR–HDAC complexes. Interestingly, the binding of SMRT and N-CoR to the catalytic HDAC3 subunit is necessary and sufficient to form an active deacetylase[24,25]. This activating role for SMRT and N-CoR requires the amino-terminal SANT1 domain[24,25]. Single amino-acid changes

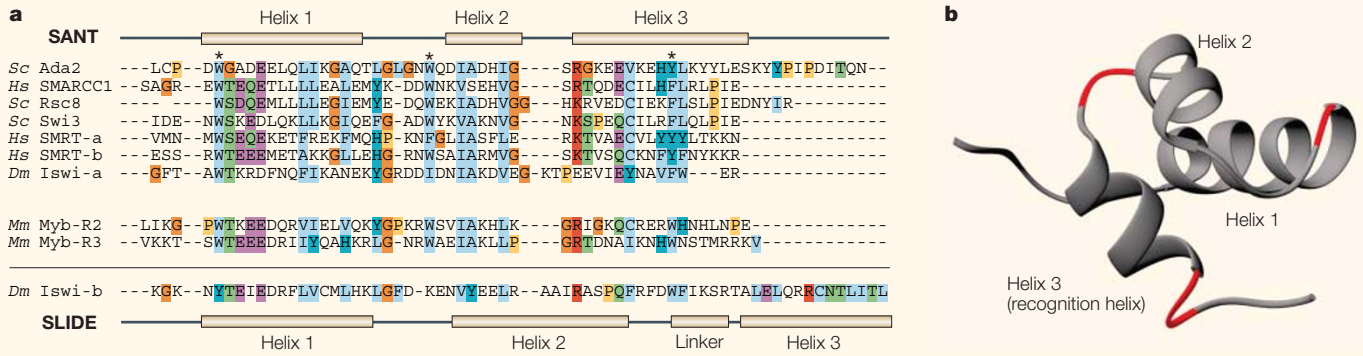


Figure 1 | **The SANT domain is a highly conserved motif that is similar to Myb DNA-binding domains. a** | SANT (switching-defective protein 3 (Swi3), adaptor 2 (Ada2), nuclear receptor co-repressor (N-CoR), transcription factor (TF)IIIB)- and SLIDE (SANT-like ISWI (imitation switch) domain)-containing sequences (including Myb repeats (R) 2 and 3) were aligned using ClustalX v1.81 (REF. 37). SANT domain and Myb sequences are shown above the line, and a SLIDE sequence is shown below the line. In the sequence alignment, columns of residues that show sequence similarity are coloured according to their respective properties (ClustalX v1.81). For the SANT domains (upper alignments) and the SLIDE domain (lower alignment), the corresponding secondary structures from the Iswi crystal structures are shown above and below the alignments, respectively. Asterisks highlight the bulky hydrophobic residues that are predicted to form the hydrophobic core of the SANT domain. Sequences are labelled on the left with the first two letters of the species name (*Dm*, *Drosophila melanogaster*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus*; *Sc*, *Saccharomyces cerevisiae*) followed by the common protein name. Ada2, adaptor 2; Rsc, remodel the structure of chromatin; SMARCC1, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin; SMRT, silencing mediator of retinoid and thyroid receptors. **b** | Ribbon diagram of a Myb DNA-binding domain that is based on the averaged solution structure of Myb R2 (Protein Data Bank accession code 1GV5)[15]. Residues in the third — or 'recognition' — α-helix make sequence-specific contacts with DNA. The three α-helices have a similar topography to the Iswi SANT domain[16].

in SANT1 disrupt the binding of HDAC3 to SMRT, which indicates that this SANT domain contributes to protein–protein interactions as well as to enzymatic activation. It is noteworthy that a SANT domain in metastasis-associated protein 2 (MTA2), which is a component of the nucleosome remodelling and deacetylase complex (NuRD), seems to contribute to the MTA2–HDAC1 interaction[26], and also that a SANT domain in co-RE1 silencing transcription factor (CoREST) is essential for HDAC1 activation[27]. This indicates a highly conserved role for some SANT domains in HDAC activation. However, it remains unclear how these SANT domains activate the catalytic HDAC subunit.

Recent studies have shown that the carboxy-terminal SANT2 domain in SMRT functions as a histone-tail-interaction domain[24]. Specifically, the isolated SANT2 domain binds to an unacetylated histone-H4 peptide, and this interaction is disrupted by tetra-acetylation of H4. Furthermore, the presence of SANT2 enhances the HDAC activity of SMRT–HDAC3 by increasing the affinity of the complex for histone tails (decreased K_m). Although the specificity of SANT2 for unacetylated tails seems like a contradiction, given that the histone substrate for SMRT is an acetylated tail, Lazar and colleagues have suggested that SANT2 might facilitate HDAC activity by stabilizing the product of the reaction and by blocking subsequent acetylation by other enzymes. In general, the ability of SANT2 to enhance tail binding by the catalytic HDAC subunit is remarkably similar to the role of the Ada2 SANT domain in Gcn5-dependent histone acetylation. The SANT2 domain cannot substitute for SANT1 in terms of HDAC binding and activation, so it seems that these two closely spaced SANT domains carry out distinct roles in order to synergistically promote and maintain histone deacetylation.

SANT and ATP-dependent chromatin remodelling. The *D. melanogaster* ISWI (imitation switch) family of ATP-dependent chromatin-remodelling enzymes is conserved from yeast to humans. One distinguishing feature of these enzymes is that their ATPase activity is only weakly stimulated by DNA and maximal activity requires that the DNA is wrapped around a histone octamer that contains histone amino-terminal tails[28,29]. In the case of Iswi, optimal ATPase activity requires a small sequence on the histone-H4 tail[30]. By contrast, other remodelling enzymes, such as SWI/SNF and Mi-2/NuRD, require only DNA for maximal ATPase activity[31].

A common structural feature of all ISWI enzymes is that they contain two closely juxtaposed SANT-like domains that are carboxy-terminal of their conserved ATPase cassette. Sequence alignments indicate that the carboxy-terminal SANT domain is considerably different from the canonical SANT sequence, and database searches with this domain find only sequences of ISWI-related proteins[16] (FIG. 1). On the basis of these properties, Grüne and colleagues called this motif SLIDE (SANT-like ISWI domain) to distinguish it from the canonical SANT domain[16]. As the histone-tail dependence of ISWI enzymes does not require further subunits[29], the SLIDE and/or SANT domains are excellent candidates for histone-interaction modules that link histone binding to ATPase activity.

Grüne and colleagues analysed the biochemical properties of recombinant Iswi derivatives that lack SLIDE, SANT or both domains[16]. Derivatives that lack the SLIDE domain are unable to bind to synthetic four-way (Holliday)-junction DNA, and their ATPase activity is insensitive to both DNA and nucleosomal DNA cofactors. By contrast, deletion of the SANT motif has no effect on four-way-junction binding, and the ATPase activity still responds weakly to DNA. Strikingly, the ATPase activity of the IswiΔSANT derivative is only poorly stimulated by nucleosomal substrates that contain amino-terminal tails. So, these data indicate a simple model in which the SLIDE domain recognizes the DNA component of nucleosomal substrates, and the SANT domain might be the histone-interaction module. Consistent with this hypothesis, the SLIDE domain has many conserved DNA-contact residues that are also found in the Myb DBD, and it has a net positive surface charge (pI = 8.3), which indicates that this motif might have maintained the DNA-binding function of Myb-related proteins[16] (FIGS 1a,2). By contrast, the SANT domain has a net negative surface charge (pI = 4.6), and several of the putative DNA-contact residues

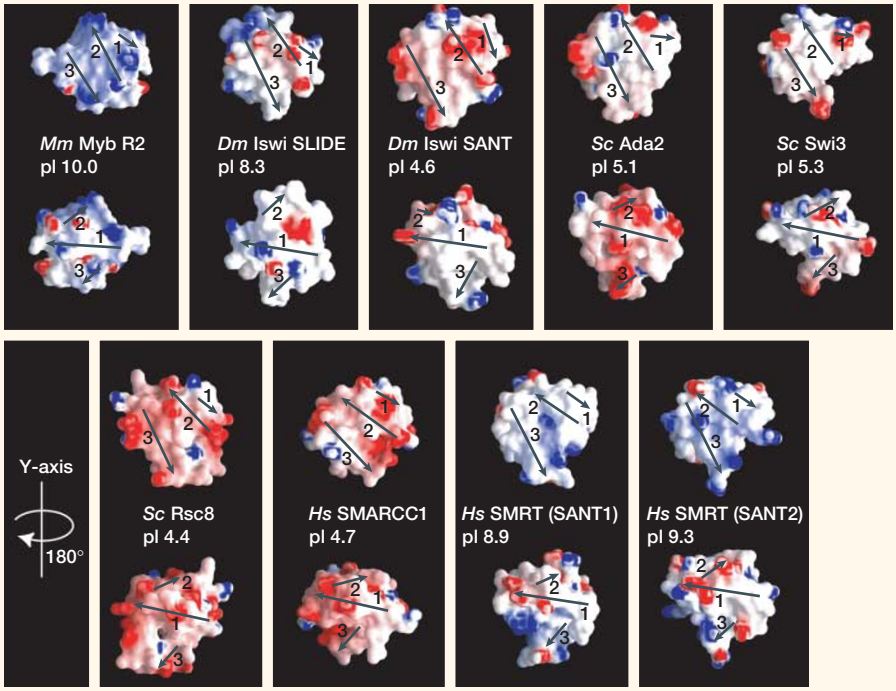


Figure 2 | **Structural modelling: the electrostatic-surface potential of selected SANT domains.** Electrostatic depictions illustrate the relative surface-charge distributions in different SANT (switching-defective protein 3 (Swi3), adaptor 2 (Ada2), nuclear receptor co-repressor (N-CoR), transcription factor (TF)IIIB)- and SLIDE (SANT-like ISWI (imitation switch) domain)-like proteins. For each protein, two electrostatic-surface diagrams show different faces of the protein (each a 180° rotation about the y-axis). The coordinates of each model/structure were aligned to Myb to place all of the surfaces in the same orientation. Arrows labelled 1, 2 and 3 identify the positions of the three main helices in each domain (FIG. 1a). Red indicates acidic residues and blue depicts basic residues, whereas white illustrates residues of neutral charge. Between the two faces, the surface diagrams are labelled with the first two letters of the species name (*Dm*, *Drosophila melanogaster*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus*; *Sc*, *Saccharomyces cerevisiae*) followed by the common protein name. Myb DNA-binding surfaces were based on the crystal structure of Myb repeat (R)2 (Protein Data Bank accession code 1GV5)[15]. *D. melanogaster* Iswi SANT and SLIDE surfaces were generated from the *D. melanogaster* Iswi structure[16]. The remaining surface diagrams were created by modelling each of the sequences using Modeller[38] and the *D. melanogaster* Iswi SANT structure as a template. The theoretical isoelectric-point (pI) values, which were calculated from the sequences that are listed in FIG. 1a, are shown (see the Compute pI/Mw tool in the Online links). The pI values correspond to the surface colouring (more acidic surfaces are predominantly red, whereas blue-tinted surfaces have a higher, more basic pI). Rsc, remodel the structure of chromatin; SMARCC1, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin; SMRT, silencing mediator of retinoid and thyroid receptors.

are acidic rather than basic[16] (FIGS 1a,2). It seems probable that SLIDE and SANT might function together to achieve both nucleosome recognition and the activation of ATPase activity, because both SLIDE and SANT are required for Iswi to bind to nucleosomes and an Iswi derivative that contains only the conserved ATPase domain shows only a basal level of ATPase activity that is not stimulated by either DNA or nucleosomal substrates.

A crucial histone-tail-effector module?

The biochemical analyses of the SANT domains from Ada2, Iswi and SMRT strongly indicate a key role for this domain in histone-tail recognition. The SANT domains in Ada2 and SMRT seem to interact primarily with unmodified histone tails[18,24]. The preference of the SMRT SANT domain for unacetylated tails indicates that local histone deacetylation might increase the affinity of SMRT SANT2 for histone tails. Interaction with unacetylated histone tails would block the binding of HATs, which would further promote HDAC activity and maintain the deacetylated state[24]. Whether other SANT domains recognize post-translationally modified histone tails is unclear at present, and this will probably be a topic of future investigation.

Importantly, SANT domains clearly have functions other than histone-tail binding, as deletion of the SANT domain can impair catalytic activity, which indicates that this motif couples substrate binding to catalysis[16,18,24]. So, how is this 'coupling' function accomplished? A simple possibility is that SANT domains bind and stabilize a high-affinity histone-tail conformation that is essential for the proper alignment (or presentation) of the substrate to the catalytic site (FIG. 3)[18]. Alternatively, as the Myb DBD undergoes a conformational shift on DNA binding[32], and given the structural conservation between the Myb DBD and SANT[16] (FIG. 1), histone-tail binding might induce a similar conformational shift in the SANT domain. Such a conformational change might provide a molecular link between substrate binding and enzyme catalysis. This model is consistent with the observation that yeast Gcn5 must undergo a compulsory conformational change in the histone-binding groove before the histone-tail substrate can be bound efficiently[33]. In the case of ISWI, the ability to undergo conformational switching might contribute to a dynamic series of enzyme–nucleosome interactions that are similar to those proposed for BRG1-dependent nucleosome remodelling[34] (BRG1 is a human SWI/SNF ATPase).

As mentioned above, bromodomains and chromodomains are extra histone-tail-binding modules that are found predominantly in subunits of chromatin-remodelling and -modification enzymes. Whereas bromodomains are commonly found in co-activator complexes and bind to acetylated histone tails, chromodomains are typically associated with transcriptional repressor complexes and a subset of these domains preferentially interacts with methylated histone tails. So, why do chromatin-remodelling enzymes contain several histone-tail-binding modules? Whereas SANT domains are often required for enzymatic activity, bromodomains and chromodomains regulate the recruitment of the enzyme to discrete chromosomal locations[11,20]. For example, histone acetylation promotes the bromodomain-mediated retention of both the SAGA HAT and SWI/SNF remodelling complexes at several promoters *in vivo*[20]. Similarly, the HP1 (heterochromatin protein 1) chromodomain targets its associated methyltransferase (SUV39H1; suppressor of variegation 3–9 homologue 1) to nucleosomes containing histone H3 that is methylated at lysine 9 (REFS 35,36). Perhaps it is the unique constellation of different histone-tail-binding modules that provides each remodelling enzyme with its diagnostic activity and localization properties (FIG. 3).

SANT as a functional predictor?

A long list of SANT-domain-containing proteins has been predicted by the SMART (simple modular architecture research tool) domain classification program (see Online

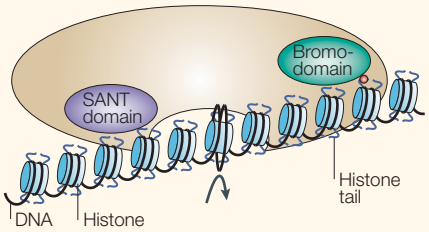


Figure 3 | **Combinatorial roles for distinct histone-binding modules.** The model depicts a theoretical multisubunit chromatin-remodelling enzyme that harbours subunits containing either a SANT (switching-defective protein 3 (Swi3), adaptor 2 (Ada2), nuclear receptor co-repressor (N-CoR), transcription factor (TF)IIIB) or a bromodomain histone-binding module. Whereas the bromodomain facilitates the recruitment and/or stability of the enzyme at a target chromosomal site that harbours acetylated histones (the acetyl group is shown as a red circle), the SANT domain couples histone-tail binding to efficient enzyme catalysis (for example, histone acetylation or ATP-dependent chromatin remodelling).

links). In addition to the SANT domains that have been shown to bind to histone tails (for example, those in Ada2 and SMRT), this list also includes the Myb and Myb-related proteins that contain genuine DBDs. Is there a more precise way to define SANT domains that have functions that are linked to chromatin? The Myb DBD is characterized by a highly electropositive surface potential (pI = ~10) that is consistent with DNA binding, whereas the Iswi SANT domain has a predominantly negative surface charge (pI = 4.6), which supports its proposed role as a basic histone-tail-recognition module (FIG. 2)[16]. Interestingly, SLIDE — a divergent SANT motif in Iswi that is required for DNA recognition — is highly basic (pI = 8.3) (FIGS 1a,2)[16]. So, the distribution of the molecular surface charge seems to be functionally descriptive and might define a canonical SANT domain (that is, one that is involved in histone-tail binding). Using this criterion, we note that the DnaJ-like chaperone — ZRF1 (zuotin-related factor 1) — contains a pair of SANT domains, one of which has a theoretical pI of 6.4 (for an electrostatic representation see SANT supplementary information in Online links). The presence of such a domain in ZRF1 might indicate that its chaperone activity is coupled to chromatin. Alternatively, the ZRF1 SANT domain might function as a molecular sensor, similar to that described above for Gcn5, that couples substrate binding to enzyme catalysis through a compulsory conformational change.

To further evaluate whether surface-charge distribution is a valid predictor of histone-binding SANT domains, the Iswi crystal structure coordinates were used to build further electrostatic molecular models for a representative set of SANT-domain-containing proteins (FIG. 2). Strikingly, the SANT domains of yeast Swi3, Rsc8 and Ada2, and of human SMARCC1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin — the orthologue of yeast Swi3) have acidic surfaces overall (pIs of 5.3, 4.4, 5.1 and 4.7, respectively; FIG. 2), which is analogous to the Iswi SANT domain. This is consistent with the histone-tail-binding properties of the Ada2 SANT domain, and indicates that the SANT domain has an analogous role in SWI/SNF- and RSC-complex function.

However, analysis of the SANT domains of N-CoR and SMRT indicates that the overall surface-charge distribution is not a sufficient criterion for classifying SANT domains. For example, both SANT domains of SMRT have theoretical pIs that are close to that of the Myb DBD (that is, pIs of 8.9 and 9.3 for SANT1 and SANT2, respectively; FIG. 2), even though SANT2 binds to histone tails *in vitro*. However, in contrast to the Myb DBD, which tends to have a uniform distribution of positively charged amino acids, both the SANT1 and SANT2 domains have a clear asymmetry in their charge distribution, and have large patches of acidic residues on their surface (FIG. 2). These acidic patches are excellent candidates for the histone-binding surface. So, whereas overall charge distribution can provide a quick assessment of histone-binding ability (that is, a pI <7 indicates that the domain might bind histones), a detailed electrostatic-surface analysis can identify chromatin-related functions for other SANT domains, as well as identify asymmetric patches of charged residues that could function as targets for mutagenesis and for subsequent functional studies.

Conclusion

Although SANT domains show remarkable sequence and structural similarity to the DBD of Myb, it is now clear that SANT has evolved a function that is distinct from DNA binding. Biochemical analyses of the Ada2, Iswi and SMRT SANT domains, coupled with electrostatic modelling, support the view that SANT domains function as histone-binding modules. We note, however, that the acidic surface that is diagnostic of the SANT domain might also provide a binding surface for other highly basic protein domains. For example, some SANT domains might interact with the basic carboxy-terminal tails of linker histones or other non-histone proteins such as mammalian HMG1 (high mobility group protein 1) or yeast Sin1 (Swi-independent). If the binding of a target peptide to SANT domains does indeed induce a conformational change, then perhaps SANT domains have numerous roles as 'molecular sensors'.

Laurie A. Boyer and Robert R. Lateck are at the Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA.

Craig L. Peterson is part of the Program in Molecular Medicine, University of Massachusetts Medical School, Plantation Street, Worcester, Massachusetts 01065, USA.
Correspondence to C.L.P
e-mail: craig.peterson@umassmed.edu
doi:10.1038/nrm1314

1. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260 (1997).
2. Luger, K. & Richmond, T. J. The histone tails of the nucleosome. *Curr. Opin. Genet. Dev.* **8**, 140–146 (1998).
3. Hansen, J. C., Tse, C. & Wolffe, A. P. Structure and function of the core histone N-termini: more than meets the eye. *Biochemistry* **37**, 17637–17641 (1998).
4. Marmorstein, R. Protein modules that manipulate histone tails for chromatin regulation. *Nature Rev. Mol. Cell Biol.* **2**, 422–432 (2001).
5. Turner, B. M. Cellular memory and the histone code. *Cell* **111**, 285–291 (2002).
6. Fry, C. J. & Peterson, C. L. Chromatin remodeling enzymes: who's on first? *Curr. Biol.* **11**, R185–R197 (2001).
7. Narlikar, G. J., Fan, H. Y. & Kingston, R. E. Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**, 475–487 (2002).
8. Dhalluin, C. *et al*. Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **399**, 491–496 (1999).
9. Jacobson, R. H., Ladurner, A. G., King, D. S. & Tjian, R. Structure and function of a human TAFII250 double bromodomain module. *Science* **288**, 1422–1425 (2000).
10. Ornaghi, P., Ballario, P., Lena, A. M., Gonzalez, A. & Filetici, P. The bromodomain of Gcn5p interacts *in vitro* with specific residues in the N terminus of histone H4. *J. Mol. Biol.* **287**, 1–7 (1999).
11. Fischle, W. *et al*. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev.* **17**, 1870–1881 (2003).
12. Min, J., Zhang, Y. & Xu, R. M. Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. *Genes Dev*. **17**, 1823–1828 (2003).
13. Aasland, R., Stewart, A. F. & Gibson, T. The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. *Trends Biochem. Sci.* **21**, 87–88 (1996).
14. Ogata, K. *et al*. Solution structure of a specific DNA complex of the Myb DNA-binding domain with cooperative recognition helices. *Cell* **79**, 639–648 (1994).
15. Tahirov, T. H. *et al*. Crystals of ternary protein–DNA complexes composed of DNA-binding domains of c-Myb or v-Myb, C/EBPα or C/EBPβ and tom-1A promoter fragment. *Acta Crystallogr. D* **57**, 1655–1658 (2001).
16. Grüne, T. *et al*. Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. *Mol. Cell* **12**, 449–460 (2003).
17. Barbaric, S., Reinke, H. & Horz, W. Multiple mechanistically distinct functions of SAGA at the PHO5 promoter. *Mol. Cell. Biol.* **23**, 3468–3476 (2003).
18. Boyer, L. A. *et al*. Essential role for the SANT domain in the functioning of multiple chromatin remodeling enzymes. *Mol. Cell* **10**, 935–942 (2002).
19. Sterner, D. E., Wang, X., Bloom, M. H., Simon, G. M. & Berger, S. L. The SANT domain of Ada2 is required for normal acetylation of histones by the yeast SAGA complex. *J. Biol. Chem.* **277**, 8178–8186 (2002).
20. Hassan, A. H. *et al*. Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. *Cell* **111**, 369–379 (2002).
21. Marcus, G. A., Silverman, N., Berger, S. L., Horiuchi, J. & Guarente, L. Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adaptors. *EMBO J.* **13**, 4807–4815 (1994).

22. Sterner, D. E. *et al*. Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. *Mol. Cell. Biol.* **19**, 86–98 (1999).
23. Elfring, L. K. *et al*. Genetic analysis of brahma: the *Drosophila* homolog of the yeast chromatin remodeling factor SWI2/SNF2. *Genetics* **148**, 251–265 (1998).
24. Yu, J., Li, Y., Ishizuka, T., Guenther, M. G. & Lazar, M. A. A SANT motif in the SMRT corepressor interprets the histone code and promotes histone deacetylation. *EMBO J.* **22**, 3403–3410 (2003).
25. Guenther, M. G., Barak, O. & Lazar, M. A. The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. *Mol. Cell. Biol.* **21**, 6091–6101 (2001).
26. Humphrey, G. W. *et al*. Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1. *J. Biol. Chem.* **276**, 6817–6824 (2001).
27. You, A., Tong, J. K., Grozinger, C. M. & Schreiber, S. L. CoREST is an integral component of the CoREST-human histone deacetylase complex. *Proc. Natl Acad. Sci. USA* **98**, 1454–1458 (2001).
28. Georgel, P. T., Tsukiyama, T. & Wu, C. Role of histone tails in nucleosome remodeling by *Drosophila* NURF. *EMBO J.* **16**, 4717–4726 (1997).
29. Corona, D. F. *et al*. ISWI is an ATP-dependent nucleosome remodeling factor. *Mol. Cell* **3**, 239–245 (1999).
30. Clapier, C. R., Nightingale, K. P. & Becker, P. B. A critical epitope for substrate recognition by the nucleosome remodeling ATPase ISWI. *Nucl. Acids Res.* **30**, 649–655 (2002).
31. Boyer, L. A. *et al*. Functional delineation of three groups of the ATP-dependent family of chromatin remodeling enzymes. *J. Biol. Chem.* **275**, 18864–18870 (2000).
32. Zargarian, L. *et al*. Myb-DNA recognition: role of tryptophan residues and structural changes of the minimal DNA binding domain of c-Myb. *Biochemistry* **38**, 1921–1929 (1999).
33. Langer, M. R., Tanner, K. G. & Denu, J. M. Mutational analysis of conserved residues in the GCN5 family of histone acetyltransferases. *J. Biol. Chem.* **276**, 31321–31331 (2001).
34. Narlikar, G. J., Phelan, M. L. & Kingston, R. E. Generation and interconversion of multiple distinct nucleosomal states as a mechanism for catalyzing chromatin fluidity. *Mol. Cell* **8**, 1219–1230 (2001).
35. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120 (2001).
36. Bannister, A. J. *et al*. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124 (2001).
37. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* **25**, 4876–4882 (1997).
38. Sali, A., Potterton, L., Yuan, F., van Vlijmen, H. & Karplus, M. Evaluation of comparative protein modeling by MODELLER. *Proteins* **23**, 318–326 (1995).

Acknowledgements
We wish to thank M. Lazar, P. Becker and C. Müller for communicating results before publication. We are particularly grateful to C. Müller for the early release of the crystal coordinates of the Iswi SANT domain, which allowed us to carry out our electrostatic analyses.

Competing interests statement
The authors declare that they have no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
FlyBase: http://flybase.bio.indiana.edu/
ISWI
Interpro: http://www.ebi.ac.uk/interpro/
H2A | H2B | H3 | H4 | Myb DBD
Swiss-Prot: http://us.expasy.org/sprot/
BRG1 | CoREST | HDAC3 | HMG1 | MTA2 | N-CoR | SMRT | TFIIIB
***Saccharomyces* genome database:**
http://www.yeastgenome.org/
Ada2 | Gcn5 | Rsc8 | Swi3

FURTHER INFORMATION
Compute pI/Mw tool: http://us.expasy.org/tools/pi_tool.html
SANT supplementary information:
http://jura.wi.mit.edu/jaenisch/sant/
SMART: http://smart.embl-heidelberg.de
Access to this interactive links box is free online.