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REVIEW ARTICLE

Epigenetic virtues of chromodomains

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Abstract

The chromatin organization modifier domain (chromodomain) was first identified as a motif associated with chromatin silencing in *Drosophila*. There is growing evidence that chromodomains are evolutionary conserved across different eukaryotic species to control diverse aspects of epigenetic regulation. Although originally reported as histone H3 methyllysine readers, the chromodomain functions have now expanded to recognition of other histone and non-histone partners as well as interaction with nucleic acids. Chromodomain binding to a diverse group of targets is mediated by a conserved substructure called the chromobox homology region. This motif can be used to predict methyllysine binding and distinguish chromodomains from related Tudor "Royal" family members. In this review, we discuss and classify various chromodomains according to their context, structure and the mechanism of target recognition.

Keywords: Lysine methylation, histone code, chromatin signaling, chromatin remodeling, nucleosome recognition, binary switches, tudor royal family

Introduction

A genomic revolution was launched with the completion of the human genome project a decade ago that led to new paradigms for understanding gene expression (Schmutz *et al.*, 2004; Consortium, 2004). Due to complex processes ranging alternative splicing and post-translational modifications, there are estimated to be over 1 million different human protein forms among 21,000 genes. Central to these processes are distinct chromatin transactions yielding epigenetic phenomena (Bonasio *et al.*, 2010). The unit particle of chromatin is the nucleosome consisting of conserved histones wrapped by a DNA superhelix (Luger *et al.*, 1997; Khorasanizadeh, 2004). Signaling to nucleosomes occurs via post-translational modifications of histones and DNA cytosine methylations (Bannister and Kouzarides, 2011; Feng *et al.*, 2010b).

A histone code has been proposed that states distinct modifications of histones act sequentially or in combination to recruit proteins, and bring about downstream regulatory events of gene expression (Strahl and Allis, 2000). Chromodomains are the founding class of histone code methyllysine "readers". The methyllysine histone codes are also subjects of other "readers" through modular interactions of Tudor, MBT, PWWP, PHD finger, Ankyrin repeats, and WD repeats (Taverna *et al.*, 2007). These effector modules are integral to control of transcription that responds to epigenetic initiators from environmental and developmental cues. Epigenetic effects influence common human diseases such as cancers, heart disease, diabetes and psychiatric disorders from patient to patient. A new study shows the most well-known chromodomain for reading a methyllysine in repressive chromatin, the Polycomb chromodomain, when fused to transcription factors can synthetically activate transcription in human cells (Haynes and Silver, 2011). A better understanding of functions ascribed to chromodomains should facilitate translational research and the discovery of therapeutic or diagnostic tools.

Historically, the chromodomain was discovered as a motif responsible for imprinting a determined state into the chromatin of *Drosophila*, present in the Heterochromatin protein 1 (HP1) and Polycomb (Pc)

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proteins (James and Elgin, 1986; Paro, 1990; Paro and Hogness, 1991; Aasland and Stewart, 1995). Subsequently, the three-dimensional structures corresponding to the HP1 chromodomain and chromo shadow domain revealed a conserved fold consisting of three anti-parallel β-strands followed by an α-helix (Ball *et al.,* 1997a, Brasher *et al.,* 2000). Their modularity facilitated the prediction of chromodomain and chromo shadow domain from amino acid sequences using SMART or InterPro tools (Letunic *et al.,* 2009; Hunter *et al.,* 2009) (Figure 1). The chromodomain is a hallmark of eukaryotic species, with the exception of a few cyanobacteria that encode

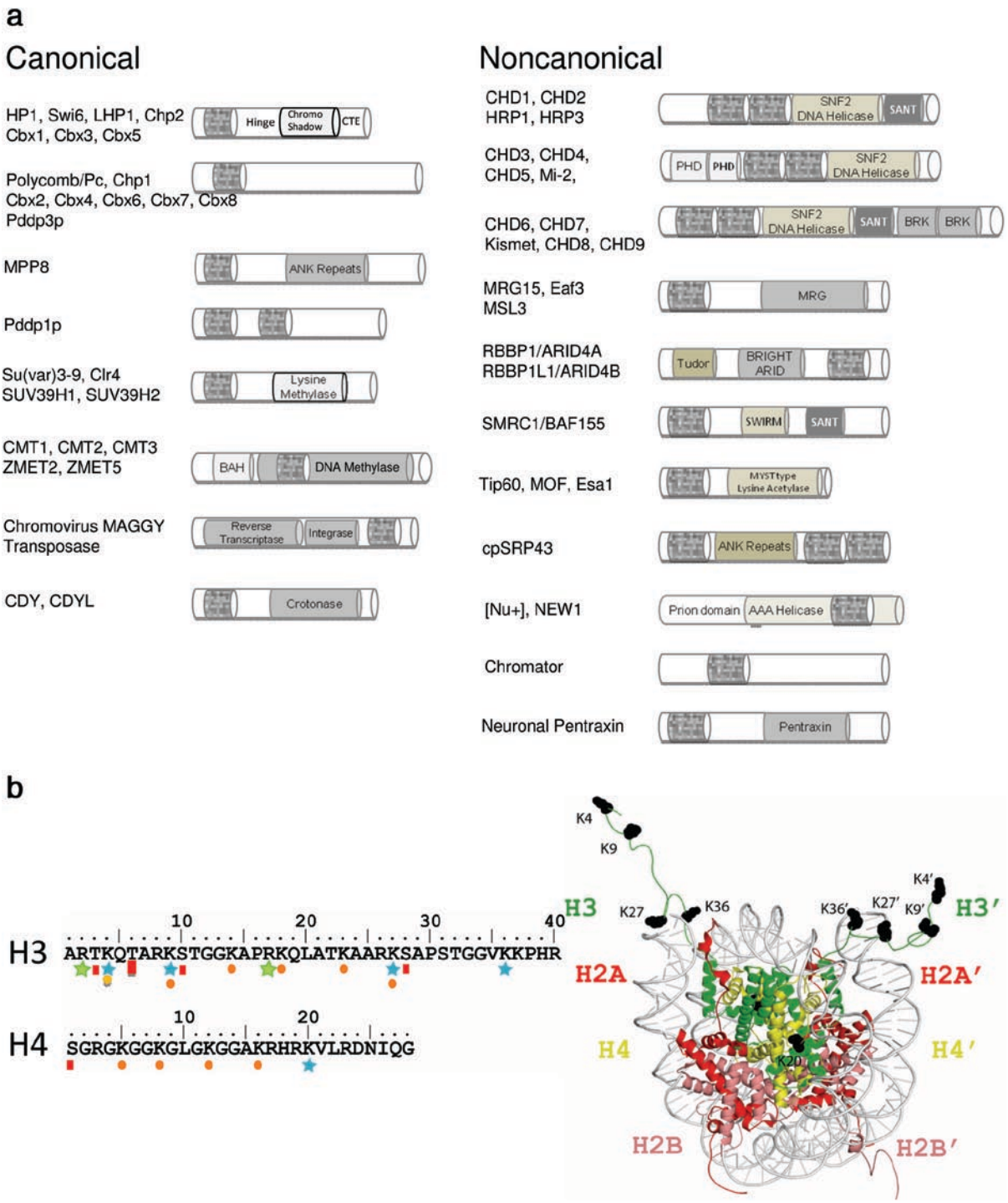

Figure 1. Diverse chromodomains recognize methyl-lysine marks in H3 and H4 tails. (a) Composition of chromodomain proteins, and their classification into canonical and noncanonical polypeptides. Chromodomain module is represented with a hatched gray rod. (b) Schematic of the absolutely conserved histone H3 and H4 tails and their locations in the nucleosome core particle. Sites of modifications with methylation of lysine (dark gray), methylation of arginine (light gray), phosphorylation of threonine (black square) and acetylation of lysines (gray circle) are labeled in histone tail sequences.

Table 1. Prevalence of chromodomain proteins in selected eukaryotic species derived from SMART database.

Species	Total number of chromodomain proteins
Nostoc punctiforme	1
Saccharomyces cerevisiae	4
Schizosaccharomyces pombe	9
Tetrahymena thermophile	18
Caenorhabditis elegans	22
Arabidopsis thaliana	23
Drosophila melanogaster	25
Homo sapiens	55

one chromodomain-containing protein. According to SMART database, the human and *Drosophila* genomes encode 55 and 25 chromodomain-containing polypeptides, respectively (Table 1) (Letunic *et al.,* 2009). For certain chromodomain proteins, there are multiple variants (e.g., 5 *Drosophila* HP1s, HP1a, HP1b, HP1c, HP1d and HP1e (Vermaak *et al.,* 2005) vs. 3 mammalian HP1 variants HP1α, HP1β and HP1γ).

A range of functions is associated with chromodomains. A majority specifically associate with sites of lysine-methylation along the absolutely conserved histone H3 and H4 tails (histone methyl-marks) (Figure 1 and Table 2). The interaction of a chromodomain with a histone methyl-mark is sensitive to the degree of methylation as well as to the presence of adjacent histone modifications such as phosphorylation of serines/threonine, methylation of arginine or acetylation of lysine. These are referred to as binary switches in histone biology (Fischle *et al.,* 2003a). In addition, chromodomains can associate with RNA and DNA in a sequence-selective manner, whereas the chromo shadow domain binds peptides with a central valine (Smothers and Henikoff, 2000). The dissociation constants measured display a wide μM range, suggesting these effectors can mediate versatile functions. Positive and negative regulations of these interactions have been associated with phosphorylation sites in chromodomain proteins as discussed below.

Herein, we classify chromodomains based on their structure and mechanism of target recognition (Figure 1a). Those with high sequence homology to the HP1 chromodomain will be referred to as canonical. They form selective interactions with a conserved lysine-methylated motif (ARKS) originally found in the H3 tail at H3K9 and H3K27 sites (Figure 1b), but also are present within other proteins (Table 3). Canonical chromodomains are abundantly encoded in mammals (17 polypeptides in total), whereas lower eukaryotes use far fewer (Letunic *et al.,* 2009). In budding yeast, the methylations of H3K9 and H3K27 are both absent (Millar and Grunstein, 2006), hence HP1 protein is not among the total of four chromodomain proteins encoded. In contrast, the fission yeast implements H3K9 methylation, and encodes four polypeptides (Clr4, Swi6, Chp1 and Chp2) that are able to bind this mark among a total of 8 chromodomain polypeptides encoded (Zhang *et al.,* 2008; Schalch *et al.,* 2009).

The ARKS/T binding chromodomains have been found in a subset of so-called chromoviruses (a form of transposases) that target the H3K9 methyl-mark. Similar chromodomain-containing transposases are encoded in plants, but none have been reported in mammals (Gorinsek *et al.,* 2004; Gorinsek *et al.,* 2005). Numerous other chromodomains have been reported to recognize different targets along the histone tails, e.g., the H3K4, H3K36 and H4K20 methyl-marks (Table 3). Often, these chromodomains have amino and carboxyl-terminal extensions and/or internal inserts. A significant number of chromodomains display no interaction with methylated peptides. Some are associated with nucleic acid recognition, and others may recognize unmethylated peptides. We will refer to all these chromodomains as noncanonical (Figure 1a).

The chromobox homology motif harbors critical aromatic residues for methyllysine recognition. We discuss the use of this motif as a prediction tool for the identification of chromodomains and their functions. We conclude with a comparison of chromodomains with Tudor, MBT, PWWP and Agenet domains collectively coined as the Royal family (Maurer-Stroh *et al.,* 2003). As noncanonical chromodomains often fold like the Tudor domain, we conclude by evaluating attributes of Tudor domains.

Canonical chromodomains

HP1 and Polycomb chromodomains

In *Drosophila*, HP1 and Polycomb chromodomains direct the localization of distinct chromatin repressive complexes to the H3K9 and H3K27 methyl-marks, respectively (Jacobs *et al.,* 2001; Fischle *et al.,* 2003b). In mammals, the Polycomb protein has expanded to five (Cbx2, Cbx4, Cbx6, Cbx7 and Cbx8) proteins; the Cbx1, Cbx3 and Cbx5 correspond to the HP1 variants. The five Polycomb variants have roles in assembly and function of the various forms of the polycomb repressive complex 1 (PRC1) that regulates tissue-specific development and disease (Simon and Kingston, 2009). A common mechanism of methylated histone tail recognition is achieved by the HP1 and Polycomb chromodomains (Figure 2).

Canonical chromodomains accommodate the histone H3 peptide in a groove between β1 and β3 strands and complete a β-sandwich architecture (Jacobs *et al.,* 2001; Nielsen *et al.,* 2002; Jacobs and Khorasanizadeh, 2002; Kaustov *et al.,* 2011) (Figure 2a). The binding involves the insertion of the methyllysine moiety into a groove lined by three aromatic residues coined as an aromatic cage (Jacobs and Khorasanizadeh, 2002). In this manner, the disordered histone H3 tail adopts an ordered conformation upon binding to the chromodomain, whereas the structure and dynamics of the chromodomain do not change upon binding. Their interaction requires the presence of methyllysine at H3K9 and formation of cation-π interactions with delocalized electrons of three conserved aromatic residues (Figure 2b). HP1 forms robust interactions with mono,

Table 2. Histone and non-histone targets reported for chromodomain proteins.

Recruiting Histone Mark	Localization/Function	Chromodomain
H3K4me3	Transcription start sites, transcription activity (Barski *et al.,* 2007, Santos-Rosa *et al.,* 2002, Schneider *et al.,* 2004)	Human CHD1 (Flanagan *et al.,* 2005), CHD2, CHD8 (Flanagan *et al.,* 2007, Rodriguez-Paredes *et al.,* 2009), Eaf3 (Xu *et al.,* 2008), CHD7 (Takada *et al.,* 2007)
H3K4me2	Active or poised chromatin, transcription activity (Bernstein *et al.,* 2005, Liang *et al.,* 2004)	
H3K4me1	Gene enhancers, transcription activity (Heintzman *et al.,* 2007)	CHD7 (Bajpai *et al.,* 2010, Schnetz *et al.,* 2009)
H3K9me2/3	Pericentric heterochromatin, transcription activity or silencing (Barski *et al.,* 2007, Bernstein *et al.,* 2005, Liang *et al.,* 2004)	HP1 (Bannister *et al.,* 2001, Jacobs and Khorasanizadeh, 2002), LHP1 (Zhang *et al.,* 2007), MPP8 (Quinn *et al.*), Chp1 (Schalch *et al.,* 2009), Swi6 (Nakayama *et al.,* 2001, Sadaie *et al.,* 2008), Chp2 (Sadaie *et al.,* 2008), Clr4 (Zhang *et al.,* 2008), MAGGY (Kordis, 2005), Tip60 (Sun *et al.,* 2009), CHD7 (Takada *et al.,* 2007), Pdd1/3p (Taverna *et al.,* 2002), Cbx2/4/7 (Bernstein *et al.,* 2006), Su(var)3-9 (Jacobs *et al.,* 2004), CMT3 (Lindroth *et al.,* 2004)
H3K9me1	Pericentric heterochromatin, transcription activity (Peters *et al.,* 2003)	CDYL, CDY (Fischle *et al.,* 2008)
H3K27me2/3	Body of genes, transcription silencing (Cao *et al.,* 2002)	LHP1 (Zhang *et al.,* 2007), Pc (Fischle *et al.,* 2003b, Min *et al.,* 2003), CBX2/7 (Bernstein *et al.,* 2006)
H3K27me1	Pericentric heterochromatin, transcription silencing (Heintzman *et al.,* 2007)	-
H3K36me2/3	Body and 3'- end of genes, transcription activity (Lee *et al.,* 2007)	MRG15 (Zhang *et al.,* 2006), Eaf3 (Sun *et al.,* 2008, Xu *et al.,* 2008), MSL3 (Sural *et al.,* 2008)
H4K20me2/3	Chromosomal integrity, transcription silencing (Lan and Shi, 2009, Mikkelsen *et al.,* 2007)	-
H4K20me1	Body of genes, transcription elongation or silencing (Karachentsev *et al.,* 2007, Vakoc *et al.,* 2006)	MSL3 (Kim *et al.,* 2010)
Antagonizing Histone Mark	Localization/Function	Chromo
H3T3ph	Mitotic centromeres, mitosis regulation (Dai *et al.,* 2005, Rosasco-Nitcher *et al.,* 2008)	Human CHD1 (Flanagan *et al.,* 2005)
H3R2me2asym	Body and 3'- end of genes(Guccione *et al.,* 2007)	Human CHD1 (Flanagan *et al.,* 2005)
H3S10ph	Transcription activity, chromosome condensation and segregation during mitosis (Hendzel, 1997, Johansen and Johansen, 2006, Wei *et al.,* 1999)	HP1 (Fischle *et al.,* 2005, Jacobs and Khorasanizadeh, 2002)
H4K16ac	Genomewide distribution, transcription activity (Wang *et al.,* 2008)	MSL3 (Kim *et al.,* 2010)
Recruiting Chromatin Factor	Localization/Function	ChSh
EMSY	DNA-damage response and chromatin remodeling (Hughes-Davies *et al.,* 2003)	HP1 (Ekblad *et al.,* 2005, Huang *et al.,* 2006)
CAF-1	Nucleosome assembly during DNA replication and repair (Gaillard *et al.,* 1996, Kaufman *et al.,* 1995, Murzina *et al.,* 1999, Smith and Stillman, 1989)	HP1 (Thiru *et al.,* 2004)
PIWI	RNA silencing complex/binds to piRNAs (Brennecke *et al.,* 2007, Vagin *et al.,* 2006)	HP1 (Brower-Toland *et al.,* 2007)
DIM-2	Heterochromatin/DNA methylation (Kouzminova and Selker, 2001, Tamaru and Selker, 2001)	*N. crassa* HP1 (Honda and Selker, 2008)
Histone H3	Activation of JAK2 by chromosomal translocations or point mutations in haematological malignancies (Dawson *et al.,* 2009)	HP1a (Dawson *et al.,* 2009)

Table 3. Human proteins that contain an ARKS/T motif are classified according to function with their accession codes listed in parenthesis. These may be putative physiological targets of a variety of lysine methyltransferases.

Nuclear functions	Zinc finger	Transcription regulation	Histone methyltransferase/ demethylase	Adapter	Chromosomal stability/ DNA replication	Catalytic activity	Polymerase-associated
	ZFHX4 (Q86UP3)	ADNP (Q9H2P0)	NSD1 (Q96L73)	TRRAP (Q9Y4A5)	MCM5 (P33992)	BMP2K (Q9NSY1)	BN51 (P05423)
	ZNF231 (Q9UPA5)	TSHZ3 (Q63HK5)	NSD3 (Q9BZ95)	ATF-IP (Q6VMQ6)	DPOG1 (P54098)	PRP4 (Q13523)	RPA194 (O95602)
	ZNF449 (Q6P9G9)		G9a (Q96KQ7)	ACRC (Q96QF7)	LIG1 (P18858)	EXD2 (Q9NVH0)	POLR1A (B7ZKR9)
	ZNF484 (Q5JVG2)	Sp110 (Q9HB58)	MLL2 (O14686)	CLIP4 (P57075)	GTBP (P52701)	NTHL1 (P78549)	DHTKD1 (Q96HY7)
	ZNF518A (Q6AHZ1)	MR (P08235)	MLL5 (Q8IZD2)	WDR62 (O43379)	FAC1 (Q9NVI1)	EST1A (Q86US8)	
	ZNF644 (Q9H582)	GR (P04150)	EHMT1 (Q9H9B1)		Repo-Man (Q69YH5)	ATAD2B (Q9ULI0)	
	ZNF707 (Q96C28)	BBX (Q8WY36)	UTX (O15550)		RBM12 (Q9NTZ6)	CSTF-77 (Q12996)	
	ZNF839 (A8K0R7)	BCL6 (E3UVQ2)	UTY (O14607)			NEIL3 (Q8TAT5)	
	ZBED6 (P86452)						
		CTCF (P49711)					
		SPT16 (Q9Y5B9)					
		PAS3 (Q8IXF0)					
		RORa-2 (P35398)					
	Catalytic activity/ signaling	Membrane-associated	Cell migration/adhesion/ cytoskeleton/apoptosis	Transport	Cell cycle/microtubule-associated	Development/ differentiation/disease	
	GVINP1 (Q7Z2Y8)	DMXL2 (Q8TDJ6)	LAMA4 (Q16363)	LTC4 (P33527)	KIF17 (Q9P2E2)	CHRD (Q9H2X0)	
	VIP1 (Q6PFW1)	FAM171A1	BOC (Q9BWV1)	AFTPH (Q6ULP2)	GTSE-1 (Q9NYZ3)	BMP-1 (P13497)	
	DPDE3 (Q08499)	(Q5VUB5)	EMILIN-4 (Q13201)	ATP13A4 (Q4VNC1)	CLASP2 (O75122)	SAP-97 (Q12959)	
	VIP2 (O43314)	PRTG (Q2VWP7)	ADB2 (Q13349)	GABRG2 (P18507)	DP-150 (Q14203)	NRK (Q7Z2Y5)	
	MTR (Q99707)	GCC1 (Q96CN9)	MAC2BP (Q08380)	GABRG3 (Q99928)	DNM1 (Q05193)	LNP (Q9C0E8)	
	XDH (P47989)	C9orfl44B (Q6ZU69)	PC-LKC (Q9BYE9)	LYST (Q99698)	Cyclin-F (P41002)	CASP-14 (P31944)	
	AP-A (Q07075)	5-HT-7 (P34969)	N-RAP (Q86VF7)	CrAT (P43155)		EDF (P08476)	
	PIK3C2B (B7ZM86)	CIP150 (Q4ADV7)	CK-73 (Q86Y46)	Synip (Q6ZWJ1)		Munc13-3 (Q8NB66)	
	ART3 (Q13508)	MUC-16 (Q8WXI7)	SYNC (Q9H7C4)	VATB (P15313)		AP-N (P15144)	
	JAK-3 (P52333)	DCST2 (Q5T1A1)	ARPC5 (O15511)	40-2-3 (Q96QD9)		NF-1 (P21359)	
	SE (Q14534)	COG2 (Q86U99)	DAAM1 (Q9Y4D1)	SNX19 (Q92543)		GRASP-1 (Q4V328)	
	USP31 (Q70CQ4)	TMC3 (Q7Z5M5)	Acinus (Q9UKV3)	CAB4 (O00305)		Ru2 (Q9UPZ3)	
	DEPTOR (Q8TB45)	TMEM68 (Q96MH6)	Carma 3 (Q9BWT7)	PN5 (Q9UI33)			
	LEI (P30740)	LPPR5 (Q32ZL2)	NLRP6 (P59044)	KCTD6 (Q8NC69)			
	TULP4 (Q9NRJ4)	GTSCR1 (Q86UQ5)	PDCD6I2 (Q8WUM4)	SKD2 (Q9UN37)			
	FBX041(Q8TF61)	OST267 (Q96RD1)	GADD34 (O75807)	HGS (O14964)			
	USPL1 (Q5W0Q7)	TMEM215 (Q68D42)	SMG7 (Q92540)	RIM 2 (Q9UQ26)			
	MR-GEF (Q92565)	JP-2 (Q9BR39)	MECI-1 (P40306)	RYR-3 (Q15413)			
	mHag HA-1 (Q92619)	DHHC-22 (Q8N966)					
	IQGAP2 (Q13576)	EPN2 (O95208)					
	DOCK1 (B2RUU3)	MMR (P22897)					
	DOCKS (Q9H7D0)	PRG-2 (Q6T4P5)					

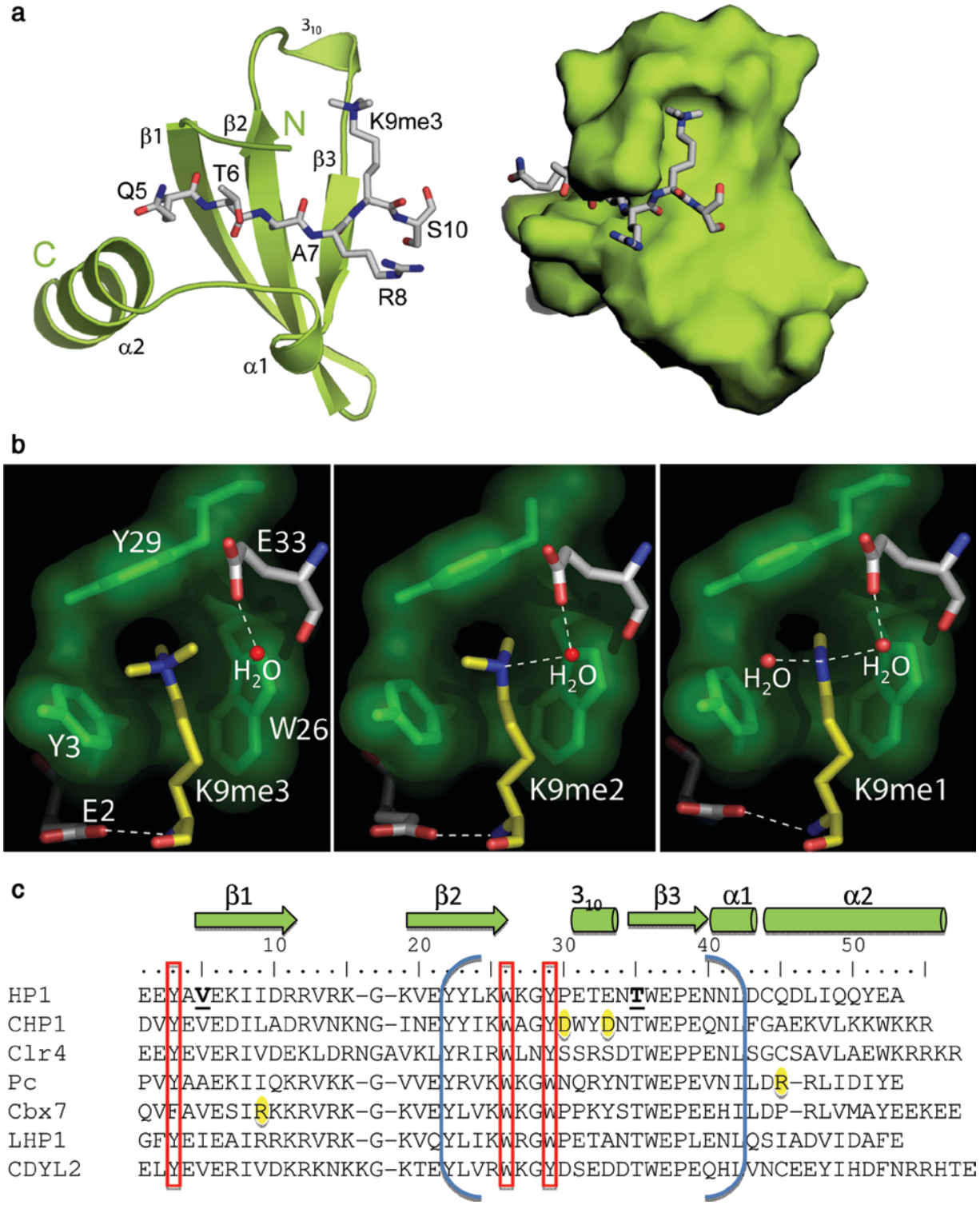

Figure 2. Structural properties of canonical chromodomains. (a) Ribbon and surface representation of the HP1 chromodomain bound to the H3K9me3 peptide (in ball and stick). (b) Differentially methylated peptide lysines form the similar cation-π interactions with the aromatic cage of the HP1 chromodomain. The free hydrogens in mono- and dimethyllysine form water-mediated hydrogen bonds (dotted lines) with the chromodomain. (c) Sequence alignment of HP1 chromodomain with other well-studied canonical chromodomains. Vertical bars depict the aromatic residues of the cage. The brackets correspond to the chromobox homology motif. In bold is the threonine that resides in the Casein Kinase II consensus sequence. Oval highlighted residues uniquely contribute to structure of complexes.

di and trimethylated H3K9 (Fischle *et al.*, 2003b, Jacobs and Khorasanizadeh, 2002), but the binding to di and trimethylation is physiologically-relevant (Grewal and Elgin, 2007). Substitution with a neutral trimethyllysine analog, the tert-butylnorleucine, results in 30-fold weaker binding to the H3K9 methyl-mark (Hughes

et al., 2007). Interestingly, a superimposable aromatic residue arrangement has been found in the human phosphatidylcholine transfer protein binding to the quaternary amine of dilinoleoyl-phosphatidylcholine (Roderick *et al.,* 2002).

Electrostatic interactions dominate in the binding of the HP1 chromodomains to the target peptide (Jacobs and Khorasanizadeh, 2002; Kaustov *et al.,* 2011). Acidic residues of the peptide groove or adjacent surfaces form polar interactions. In contrast, the Polycomb and mammalian Cbx variants rely on hydrophobic side chains at their surface to clasp the target peptide (Fischle *et al.,* 2003b, Min *et al.,* 2003; Kaustov *et al.,* 2011) (Figure 2c). Subtle amino acid substitutions in the peptide groove can lead to conformational heterogeneity in the backbone, and inability to bind peptide (Jacobs *et al.,* 2001; Kaustov *et al.,* 2011). Sequence variations among these chromodomains result in a spectrum of binding affinity towards the H3K9 and H3K27 methyl-marks (Bernstein *et al.,* 2006; Kaustov *et al.,* 2011) (Figure 2c). For example, Cbx7 binds the H3K9 and H3K27 methyl-marks with micromolar affinity whereas Cbx6 and Cbx8 associate using millimolar affinity. Sequence variation has also led to bimodal chromatin interactions. An arginine present in the β1 of Cbx7 chromodomain (Figure 2c) is crucial for non-coding RNA recognition near where H3K27 methyl-mark binds (Yap *et al.,* 2010). Another novel interaction is the oligomerization by the chromodomain of yeast Swi6 when interacting with H3K9 methylated nucleosomal substrates (Canzio *et al.,* 2011). Point mutations of the Swi6 chromodomain that increase its dimerization ability were shown to enhance yeast chromatin silencing *in vivo* (Canzio *et al.,* 2011) in agreement with a chromodomain role in spreading of heterochromatin (Nakayama *et al.,* 2001).

Chromodomain phosphorylation regulates peptide binding. Canonical chromodomains have a conserved threonine in β3 (Figure 2c) that resides in a Casein Kinase II consensus sequence. This site is phosphorylated as a response to DNA damage, and disrupts HP1 binding (Ayoub *et al.,* 2008). Phosphorylation may mediate disruption of the π-cation bonding in the aromatic cage by increasing conformational heterogeneity or electrostatic interference (Figure 2a and c). In contrast, the phosphorylation of acidic residues near the HP1 chromodomain improves peptide binding (Hiragami-Hamada *et al.,* 2011). Many Cbx proteins as well as the plant HP1/LHP1 protein do not discriminate between methylated H3K9 and H3K27 peptides. In these, phosphorylation is believed to regulate targeting (Hatano *et al.,* 2010; Zhang *et al.,* 2007; Kaustov *et al.,* 2011).

Other canonical chromodomains

In fission yeast, a Cbx-like chromodomain resides in the Chp1 protein that tethers the RNA-induced initiation of transcription gene silencing (RITS) complex to centromeres. Chp1 binds the H3K9 methyl-mark 10-fold tighter than the Swi6/HP1 chromodomain (Schalch *et al.,* 2009). There is a larger interface of the Chp1 complex (625 Å^2) as compared to *Drosophila* HP1 (521 Å^2) (Schalch *et al.,* 2009), and a zinc ion is coordinated near the aromatic cage by two aspartic acids (Figure 2c). Binding to zinc may improve conformational order and serve advantageous to peptide binding.

The mammalian M-phase phosphoprotein 8 (MPP8) affects tumor cell proliferation and invasiveness by regulating E-cadherin expression (Matsumoto-Taniura *et al.,* 1996; Bua *et al.,* 2009; Quinn *et al.,* 2010; Kokura *et al.,* 2010). MPP8 interacts with GLP and ESET, two lysine methyltransferases and the DNA methyltransferase DNMT3A (Kokura *et al.,* 2010). Its canonical chromodomain forms a specific interaction with the H3K9 methyl-mark (Chang *et al.,* 2011). Phosphorylation of Tyr and Ser/Thr sites within the chromodomain of MPP8 are thought to control interactions with the H3K9 methyl-mark (Chang *et al.,* 2011).

The canonical chromodomains in *Tetrahymena* reside in Pdd1p and Pdd3p proteins, which play a crucial role in programmed DNA elimination. Pdd1p contains two chromodomains and the first chromodomain is selective for both H3K9me3 and H3K27me3 (Taverna *et al.,* 2002; Liu *et al.,* 2007). Pdd3p has a single chromodomain and binds H3K9me3 (Taverna *et al.,* 2002; Liu *et al.,* 2007). These functions in the macronucleus, where mega base pairs of DNA often repeat sequences, are eliminated from the genome (Chalker, 2008).

Binary switch for canonical chromodomains

The interactions of canonical chromodomains with the ARKS/T-methylated peptides are significantly reduced by Ser/Thr phosphorylation in this motif, e.g., the H3S10 or H3S28 phosphorylation (Fischle *et al.,* 2005; Niessen *et al.,* 2009). Both of these marks correlate with chromodomain protein dissociation from chromatin (Niessen *et al.,* 2009). A phosphorylation near the ARKS/T, the H3T6 was recently discovered (Garske *et al.,* 2010). A significantly reduced H3K9 methyl-mark binding by mammalian HP1 variants was observed in the presence of H3T6 phosphorylation (Kaustov *et al.,* 2011). The acetylation of H3K4 may also influence peptide binding in certain cases. Extensive contacts between the Chp1 chromodomain and H3K4 were detected for the binding to the H3K9 methylated peptide (Schalch *et al.,* 2009). In yeast, the acetylation of H3K4 signals the blockage of Chp1 interaction and prevents the spreading of the RITS complex (Xhemalce and Kouzarides, 2010). The canonical chromodomain interaction with a methyllysine is therefore modulated according to multitude of adjacent modifications that may help protect these remarkably stable methylated lysine marks (Bannister *et al.,* 2002).

Enzymes with a canonical chromodomain

The suppressor of variegation 3–9 (Su(var)3–9) protein contains a SET domain that deposits the H3K9

methyl-mark in heterochromatin (Rea *et al.,* 2000; Grewal and Jia, 2007). Deletion of the chromodomain leads to catalytic inefficiency and compromises fidelity (Chin *et al.,* 2006; Eskeland *et al.,* 2004). Its homolog in *fission yeast* is Clr4, the sole H3K9 methyltransferase that is recruited to heterochromatin by the RITS complex (Zhang *et al.,* 2008; Motamedi *et al.,* 2008; Chen *et al.,* 2008). Initial targeting of Clr4 relies on siRNAs and methylated H3K9 recognition. Clr4 chromodomain binding to H3K9me3 initiates downstream K9 methylation events allowing for the stable association of the RITS complex through the Chp1 chromodomain. This allows the RNAi machinery to process more siRNAs, which reinforce a looping mechanism that further propagates heterochromatin formation (Zofall and Grewal, 2006). This machinery is sensitive to H3K4 acetylation as Clr4 and Chp1 chromodomains could not bind to the H3K9 methyl-mark via the binary methyl/acetyl switch (Xhemalce and Kouzarides, 2010).

In *Arabidopsis*, various DNA methylation strategies are used to silence genes (Feng *et al.,* 2010a). A subset of these enzymes is the three chromomethyltransferase variants, of which only CMT3 is transcribed and CMT1 and CMT2 are considered pseudogenes. These also include maize ZMET2 and ZMET5 (Papa *et al.,* 2001) and rice CMT1, CMT2 and CMT3 (Sharma *et al.,* 2009). The chromodomain is embedded between motifs I and IV of the DNA methyltransferase enzyme, and is suspected of contributing to catalysis (Figure 1a). The CMT3 chromodomain forms a specific interaction with the lysine 9-methylated histone H3 in the context of long peptides (Lindroth *et al.,* 2004). CMT3-related chromodomains have a characteristic insertion in the β1 strand suggestive of another protein interaction surface (Lindroth *et al.,* 2004). As DNA methylation has been shown to target nucleosomes (Chodavarapu *et al.,* 2010), the CMT3 chromodomain can provide a discriminatory handle and target CMT3 to selectively function in distinct heterochromatic regions.

In a rice blast fungus Magnaportha grisea, an enzyme with reverse transcriptase and integrase functions called the chromovirus MAGGY contains the canonical chromodomain (Figure 1a) (Kordis, 2005; Gao *et al.,* 2008). To verify binding to the H3K9 methyl-mark *in vivo*, this chromodomain was fused to the Tf1 retrotransposon of fission yeast. As the wild-type Tf1 does not localize to heterochromatic regions, the presence of the chimeric retrotransposon was attributed to MAGGY localization to heterochromatin. This chromovirus and others may hijack host mechanisms to integrate into the host genome using natural histone methylation marks.

The chromodomain on the Y chromosome (CDY) family of proteins are crotonases or enoyl-CoA hydratases (Hamed *et al.,* 2008) with a canonical chromodomain (Lahn and Page, 1997). Early observation that CDY catalytic motif is a histone acetyltransferase specific for the histone H4 tail has not been reproduced (Lahn *et al.,* 2002), and the modification created by this family remains elusive. Despite a high sequence identity, the CDY, CDYL, and CDYL2 chromodomains differ in their binding preferences for H3K9 and H3K27 methyl-marks as well as for methylated non-histone peptides (Fischle *et al.,* 2008; Franz *et al.,* 2009; Kim *et al.,* 2006).

Putative targets of canonical chromodomains

We discussed a variety of biological scenarios mediating the canonical chromodomain interaction with histone H3 tail. In the last few years, evidence has accumulated for the interaction of these chromodomains with other proteins via a methylated ARKS/T motif (Huang and Berger, 2008; Daujat *et al.,* 2005; Sampath *et al.,* 2007). Moreover, the consensus sequence of numerous lysine methyltransferases in the mammalian genome is the ARKS/T motif (Allis *et al.,* 2007). These observations suggest a wider role for the canonical chromodomain in cellular signaling. We prepared Table 2 to list human polypeptides containing the ARKS/T motif, and categorized them according to presumed function. We suggest these become methylated and subject of chromodomain recognition for a variety of nuclear control mechanisms (Table 2 upper row). In addition, we found many proteins that function outside of the nucleus and may be involved in signaling, membrane association, transport or microtubule organization (Table 2 lower row).

Noncanonical chromodomains

CHD double chromodomains cooperate to bind the H3K4 methyl-mark

Chromo-ATPase/helicase-DNA-binding (CHD) proteins have double chromodomains N-terminal to a SNF2-type helicase and are vital for chromatin remodeling during development (Woodage *et al.,* 1997; Flaus *et al.,* 2006; Ho and Crabtree, 2010). *Saccharomyces cerevisiae* encodes a single CHD family member, CHD1. In metazoans, the family has expanded to four CHD proteins in *Drosophila* and nine in humans, highlighting the importance of the CHD proteins to acquire new functions in more complex organisms such as transcription elongation, DNA damage repair, osteogenic differentiation, and neurogenesis (Lutz *et al.,* 2006; Rodriguez-Paredes *et al.,* 2009; Srinivasan *et al.,* 2005; Shur *et al.,* 2006a, Shur *et al.,* 2006b, Surapureddi *et al.,* 2006; Takada *et al.,* 2007; Bajpai *et al.,* 2010; Hurd *et al.,* 2010). These proteins were divided into four phylogenetic classes based on their chromodomain sequences (Flanagan *et al.,* 2007).

In CHD1 (and CHD2) protein, the modules position orthogonally using a helical linker (Flanagan *et al.,* 2005; Flanagan *et al.,* 2007) (Figure 3a). The unconserved sequence inserts block the canonical peptide binding groove in chromodomain 1, and peptide binding is redirected to the junction of the two chromodomains. Two conserved aromatic residues in chromodomain 1 form the cage for methyllysine recognition and support binding to mono, di and trimethylated H3K4 mark in metazoan CHD1 proteins (Flanagan *et al.,* 2005; Morettini *et al.,* 2011). The peptide affinity is substantially reduced with the presence

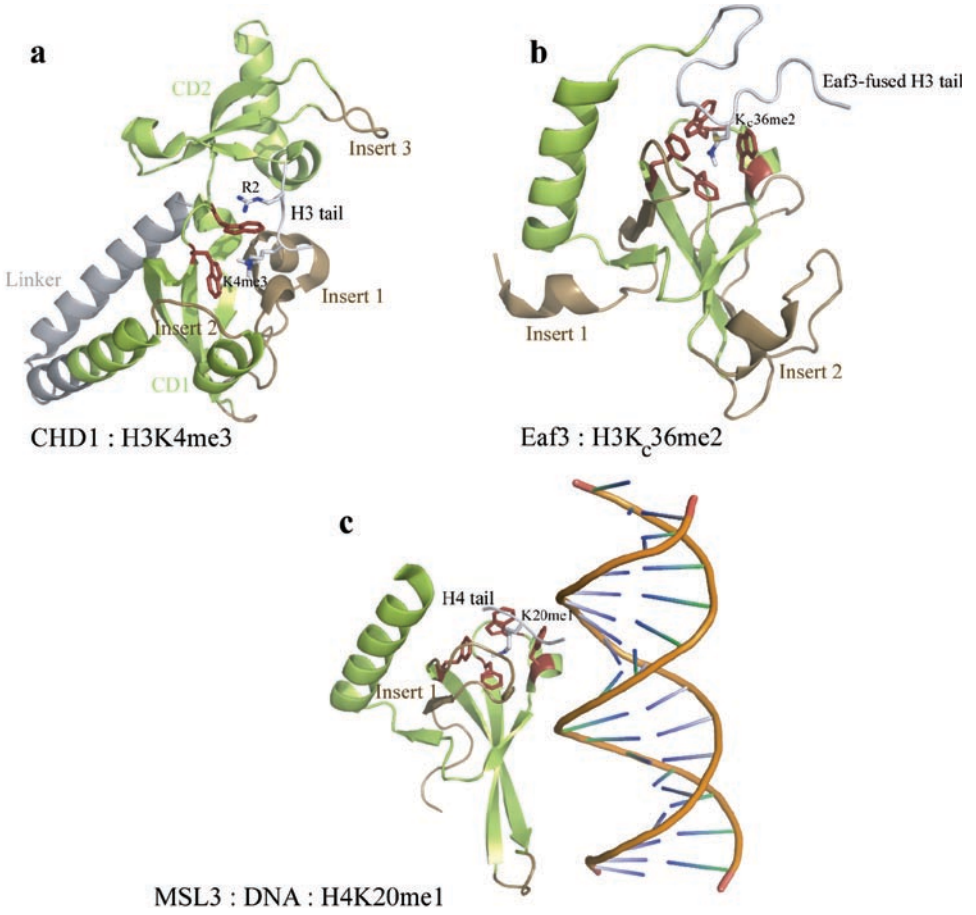

Figure 3. Sequence inserts in noncanonical chromodomains modulate binding activity. The canonical architecture of chromodomains is displayed in gray, inserts are in black, two aromatic cage residues and the peptide are drawn in ball and stick. (a) The double chromodomains of human CHD1 linked by a novel helix-turn-helix (black). (b) The solution structure of the yeast Eaf3 chromodomain with its C-terminal extension containing a methyllysine analog mimicking the binding of the H3K36 methyl-mark to its aromatic cage. (c) The MSL3 chromodomain in complex with a DNA duplex promoting its selectivity for the H4K20me1 peptide. The peptide sits on the DNA minor groove, with its methyllysine inserted in the four-residue aromatic cage.

of asymmetric dimethylation of H3R2 or phosphorylation of H3T3 despite the formation of identical chromodomain-peptide complexes with these dually-modified peptides (Flanagan *et al.,* 2005). Therefore, a methyl/methyl and a methyl/phos switch can influence CHD1 interactions. The phosphorylation of CHD proteins such as CHD2 also may affect affinity or specificity for peptides (Flanagan *et al.,* 2007). The antagonistic effect of H3T3 on H3K4me3 recognition has also been observed for TAF3 and TFIID transcription factors that do not contain a chromodomain, suggesting a broader role of binary switches in regulating chromatin structure and transcription (Varier *et al.,* 2010).

Both human and yeast CHD1 are essential for transcription elongation (Simic *et al.,* 2003; Sims *et al.,* 2007), and the mammalian CHD1 is essential for the pluripotency of embryonic stem cells (Gaspar-Maia *et al.,* 2009). In yeast, the lack of a key aromatic residue and changes in the folding of insert 1 prohibit the binding to the H3K4 methyl-mark or other histone peptides (Flanagan *et al.,* 2007; Okuda *et al.,* 2007; Sims *et al.,* 2005) (Figure 3a). Biochemical and structural studies on the yeast CHD1 recently revealed a function for chromodomain in catalysis (Hauk *et al.,* 2010). The second chromodomain was shown to contact lobe 1, whereas the inter-chromodomain linker blocked the DNA-binding surface of lobe 2 of the SNF2-helicase. It was further shown that chromodomains enhance substrate specificity of CHD1 for nucleosomes as compared to naked DNA (Hauk *et al.,* 2010). Due to the structural diversity noted for CHD proteins, chromodomains may mediate diverse regulatory functions.

Interestingly, the chromodomains of CHD7 have a unique specificity for the monomethylated H3K4 mark, and ChIP-chip studies have shown that CHD7 tracks H3K4 monomethylation patterns at enhancer motifs (Bajpai *et al.,* 2010; Schnetz *et al.,* 2009). Another study suggested CHD7 chromodomains bind the trimethylated H3K9 mark when CHD7 is recruited for osteoblastogenesis (Takada *et al.,* 2007), suggesting that CHD7 has bimodal histone specificity. CHD8 chromodomains have also been shown to bind the H3K4 methyl-mark (Rodriguez-Paredes *et al.,* 2009). By contrast, there is a group of CHD proteins related to the Mi-2 protein, which use chromodomains for DNA recognition (Bouazoune *et al.,* 2002; Kunert and Brehm, 2009).

Deletion of the Mi-2 chromodomains impairs ATPase activity and nucleosome mobilization (Bouazoune *et al.,* 2002). To compensate for the lack of histone tail binding by this class, Plant Homeodomain (PHD) fingers are shown to interact with the histone H3 tail selectively (Mansfield *et al.,* 2011; Musselman *et al.,* 2009; Shi *et al.,* 2006).

Versatility of MRG chromodomains in Eaf3/MRG15 and MSL3

Members of the chromodomain containing Morf-related gene (MRG) family are critical components of HAT and HDAC complexes that regulate global acetylation levels. The human MRG15 protein is the founding member of the MRG family, and has been associated with development, DNA repair, and chromatin remodeling (Biswas *et al.,* 2008; Cai *et al.,* 2003; Chen *et al.,* 2010; Hayakawa *et al.,* 2010; Pena and Pereira-Smith, 2007). It is a stable component of the Tip60 HAT complex and also interacts with the mSin3A HDAC complex (Cai *et al.,* 2003; Doyon *et al.,* 2004; Yochum and Ayer, 2002). The MRG15 homolog in budding yeast, Eaf3, is a member of the NuA4 HAT and Rpd3 HDAC complexes, which have opposing functions in transcriptional regulation (Eisen *et al.,* 2001; Gavin *et al.,* 2002). In *Schizosaccharomyces pombe*, altered polarity protein 13 (Alp13) is a member of the Clr6 HDAC complex. Alp13 chromodomain mutants show increased acetylation levels genome-wide implicating that similar to Eaf3, the chromodomain is critical for regulating the deacetylase activity of the complex (Nakayama *et al.,* 2003). A protein microarray screen did not identify any methyllysine substrates for chromodomains of the MRG class (Kim *et al.,* 2006), as they form weaker interactions with histone peptides.

The MRG15/Eaf3 protein associates with two opposing HAT and HDAC complexes. Although the interaction with the H3K36 methyl-mark is physiologically significant, *in vitro* affinity is in millimolar range, and the chromodomain does not discriminate among peptides with different sequences (Sun *et al.,* 2008; Xu *et al.,* 2008; Zhang *et al.,* 2006). The binding to the H3K4 methyl-mark may also be functionally relevant (Joshi and Struhl, 2005; Reid *et al.,* 2004; Sun *et al.,* 2008; Xu *et al.,* 2008). Deletion of Eaf3 chromodomain prevents Rpd3S HDAC from distinguishing methylated nucleosome substrates, and hindering deacetylation (Li *et al.,* 2007). In another context, Eaf3 chromodomain is critical for NuA4 HAT activity (Joshi and Struhl, 2005; Reid *et al.,* 2004). The MRG15 protein and the recognition of the H3K36 methyl-mark have also been noted to be crucial to alternative pre-mRNA splicing (Luco *et al.,* 2010; Luco *et al.,* 2011).

The Eaf3 and MRG15 chromodomains are structurally related and contain a four-residue aromatic cage in addition to an N-terminal β-strand, which blocks the canonical peptide recognition site (Figure 3b) (Zhang *et al.,* 2006). The methyllysine moiety was found inserted into a four-residue aromatic cage. However, the surface of interaction between the chromodomain and the peptide is not as extensive as in the canonical chromodomain (Xu *et al.,* 2008). This accounts for the weak affinity and inability to distinguish between methylated peptides *in vitro* (Xu *et al.,* 2008). Additionally, Eaf3 contains a unique insert between β2 and β3 that does not affect methyllysine recognition and is not present in the MRG15 chromodomains.

A phylogenetic analysis of the MRG family of chromodomains indicated that the human MRG15 and yeast Eaf3 chromodomains are closely related, whereas the metazoan-specific Male-Specific Lethal 3 (MSL3) is diverged. The MSL3 protein is a member of the MSL histone acetyltransferase complex responsible for H4K16 acetylation and transcription dosage compensation (Straub and Becker, 2007; Lucchesi, 2009). Initially, *in vitro* studies suggested MSL3 protein preferentially binds nucleosomes harboring the H3K36me3 methyl-mark using its chromodomain (Sural *et al.,* 2008). Subsequent studies identified MSL3 specificity for the H4K20 methyl-mark (Kim *et al.,* 2010; Moore *et al.,* 2011). MSL3 chromodomain also binds to naked DNA or nucleosome DNA (Buscaino *et al.,* 2006; Sural *et al.,* 2008). In support of this, the MSL3 chromodomain binds to GA-rich MSL recognition elements (MREs) (Alekseyenko *et al.,* 2008) with submicromolar affinity (Kim *et al.,* 2010).

The structure of the MSL3 chromodomain revealed two adjacent minor groove binding surfaces that support a cooperative DNA binding. The binding to DNA generated specificity for a histone methyl-mark, H4K20me1 peptide that bound to DNA and a four-residue aromatic cage in chromodomain (Figure 3c). The canonical binding groove found in the HP1 chromodomain is blocked with a unique sequence insert, and peptide binds within the minor groove of the DNA. The presence of H4K16 acetylation disrupts MSL3 peptide binding, indicating the usage of a methyl/acetyl switch (Kim *et al.,* 2010). A key arginine in MSL3 chromodomain helps recognize the DNA minor groove in a manner related to an arginine in the distantly-related protein Sac7d (Robinson *et al.,* 1998).

Chromodomain similarity with the MRG family (Bertram and Pereira-Smith, 2001) is a hallmark of the ARID4A/B (AT Rich Interactive Domain 4A/B) also known as RBBP-1 (Retinoblastoma Binding Protein 1) protein, a member of the mSin3A chromatin remodeling complex (Fleischer *et al.,* 2003; Lai *et al.,* 2001). ARID4A is involved in the repression of E2F-dependent transcription and cellular proliferation through its interaction with the retinoblastoma protein (Lai *et al.,* 1999a, Lai *et al.,* 1999b). It is a transcription factor important for osteoblast differentiation, genomic imprinting, and leukemic transformation (Monroe *et al.,* 2010; Wu *et al.,* 2008; Wu *et al.,* 2006). Deletion of the ARID genes regulates histone methylation levels, but chromodomain specificity has not been reported (Wu *et al.,* 2008).

Another potential MRG-related chromodomain resides in BAF155 (BRG-1 Associated Factor (BAF) protein, which is a member in some SWI/SNF chromatin remodeling complexes (Phelan *et al.,* 1999). The BAF155 protein contributes to heterochromatin formation and

chromatin remodeling during differentiation and transcription regulation (Schaniel *et al.,* 2009).

MYST family chromodomains

The MYST proteins are lysine acetlytransferases named after the founding members MOZ, YBF2/SAS3, SAS2 and Tip60 (Sapountzi and Cote, 2010; Neal *et al.,* 2000). A protein microarray screen did not identify any methyllysine substrates for chromodomains of the MYST class (Kim *et al.,* 2006). Instead, some MYST chromodomains may be involved in nucleic acid binding (Akhtar *et al.,* 2000; Shimojo *et al.,* 2008). The *Drosophila* Males-absent on the first (MOF) and the *S. cerevisiae* Essential Sas-related acetyltransferase 1 (Esa1) proteins acetylate histone H4 genomewide (Hilfiker *et al.,* 1997; Akhtar and Becker, 2000). Loss of *Mof* results in a global reduction of H4K16 acetylation, cell cycle arrest and defects in DNA damage repair (Li *et al.,* 2010b, Sharma *et al.,* 2010). *Drosophila* MOF has an unusual chromodomain structure with short β-strands (Figure 4a). Aromatic cage residues are not conserved in the MOF chromodomain and the canonical peptide binding groove is blocked by an N-terminal extension (Nielsen *et al.,* 2005). Although MOF chromodomain module did not stably bind RNA, the full-length protein requires chromodomain for binding to a non-coding RNA target (Akhtar *et al.,* 2000; Nielsen *et al.,* 2005).

Another unusual chromodomain fold is observed for the yeast Esa1 (Shimojo *et al.,* 2008). Two β-strands extend from the amino and carboxyl terminal ends of the Esa1 chromodomain, forming an anti-parallel β sheet, which is crucial for the stability of this module (Shimojo *et al.,* 2008) (Figure 4b). This structure led to the coining of an inaccurate term, the knotted-tudor domain, for the Esa1 protein (Shimojo *et al.,* 2008). We found a protein knot detection algorithm (Kolesov *et al.,* 2007) applied to the Esa1 coordinates 2RO0 did not detect any knot. Moreover, SMART and InterPro both predict presence of a chromodomain, not a tudor domain in Esa1 protein. Interestingly, the Esa1 chromodomain has been shown to interact with single strand RNA, but this is non-specifically and weak. The surface residues important for binding RNA have been shown to have a significant role for the biological function of the Esa1 protein (Shimojo *et al.,* 2008) (Figure 4b).

Tip60 (Tat-interacting protein 60 kDa) chromodomain forms another unique structure (pdb accession 2EKO) closely related to the canonical architecture, and has a two-residue aromatic cage. However, no methyllysine binding is detected for the chromodomain module (Kim *et al.,* 2006). The intact Tip60 was shown to selectively interact with the H3K9 methyl-mark in a manner dependent on the aromatic residues in its chromodomain (Sun *et al.,* 2009). Tip60 interaction with this histone mark is proposed to initiate a Tip60-dependent DNA damage response that enhances acetyltranferase activity (Sun *et al.,* 2009).

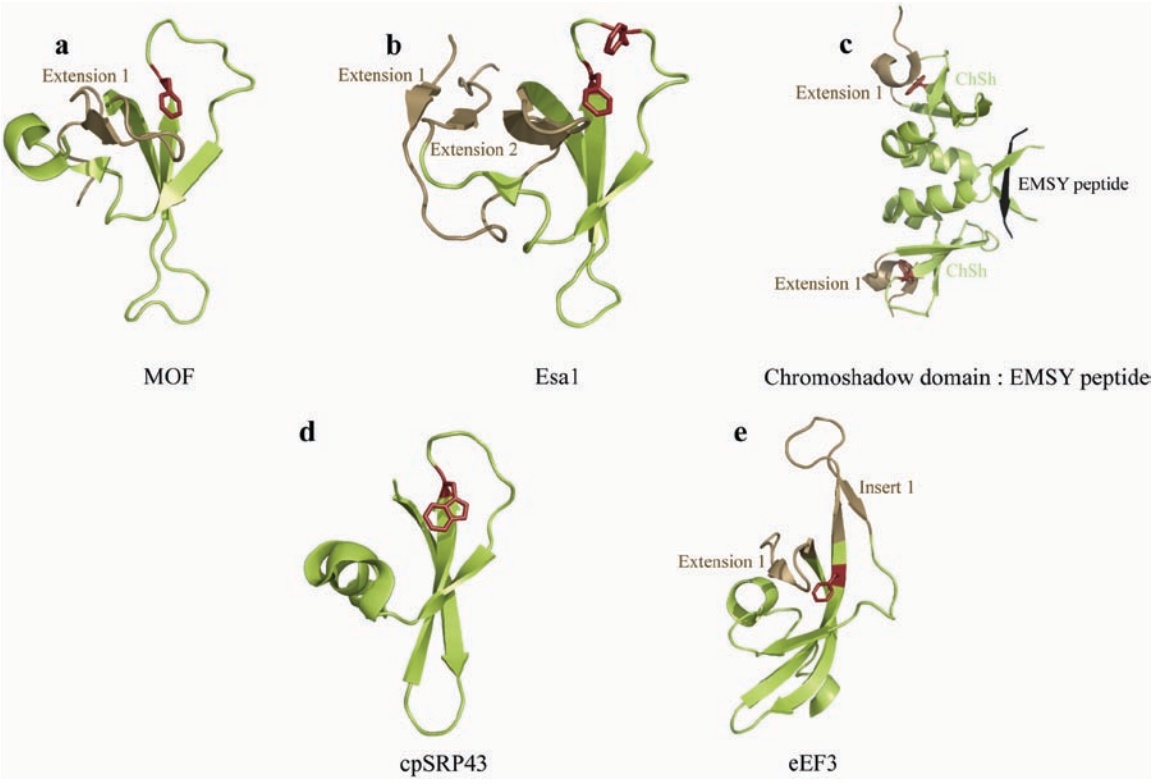

Figure 4. Noncanonical chromodomains that lack an aromatic cage. The color-coding is similar to that in Figure 3. (a) The *Drosophila* MOF chromodomain also called a chromobarrel domain. (b) Esa1 chromodomain, also called a knotted-tudor domain. (c) The human chromo shadow dimer in complex with the EMSY peptide. (d) Chromodomain 1 of cpSRP43. (e) The structurally-observed chromodomain-like domain in the eEF3.

The chromo shadow domain: a homodimer with critical carboxy terminal extension

The chromo shadow domain is found C-terminal to the chromodomain in HP1 proteins (Aasland and Stewart, 1995) (Figure 1a). The chromo shadow domain can also occur without the presence of a chromodomain, e.g. in *Drosophila* Umbrea protein, which associates with the HP1 protein (Joppich *et al.,* 2009). A number of structures reported for this domain show that it forms a symmetric homodimer stabilized by a C-terminal α-helix (Cowieson *et al.,* 2000; Brasher *et al.,* 2000; Mendez *et al.,* 2011). It exhibits a dimerization constant in low μM range (Mendez *et al.,* 2011) (Figure 4c). The structure of each monomer is highly related to that of the HP1 chromodomain. Two of the three aromatic residues associated with the chromodomain cage are substituted with hydrophobic residues. These block the canonical peptide-binding groove by forming hydrophobic interactions with a helical turn at the N-terminus. The peptide binds to the junction of dimers by forming hydrophobic interactions with a tryptophan in the C-terminus of the chromo shadow domain (Figure 4c).

The chromo shadow domain mediates HP1 association with a large list of proteins. It is known to drive the initial localization of the HP1 protein to chromatin harboring the H3K9 methyl-mark (Smothers and Henikoff, 2001; Meehan *et al.,* 2003; Lechner *et al.,* 2005). In certain regions of chromatin, it mediates assembly of DNA methylation machinery (Smallwood *et al.,* 2007; Honda and Selker, 2008). The HP1α chromo shadow domain interacted with an H3K36-specific demethylase, and suggested H3K36 demethylation is linked with heterochromatin assembly (Lin *et al.,* 2008). The HP1α chromo shadow domain also interacted with the histone H3 core region near residue 41, and binding was neutralized with JAK2 phosphorylation of tyrosine 41 (Dawson *et al.,* 2009). In plant LHP1, it interacts with LIF2 protein involved in RNA processing (Latrasse *et al.,* 2011) or binds to transcription factors SVP and SCARECROW to repress the activity of their target genes (Cui and Benfey, 2009; Liu *et al.,* 2009).

Partner selectivity for the chromo shadow domain requires the presence of PxVxL type motifs found by a phage display library screen (Smothers and Henikoff, 2000). The three-dimensional structures of the mammalian HP1β chromo shadow domain with PxVxL peptides in CAF155 and EMSY proteins show these peptides associate with a limited surface in the C-terminus of HP1β (Table 2, Figure 4) (Thiru *et al.,* 2004; Huang *et al.,* 2006; Ekblad *et al.,* 2005). Dimerization is required for the binding to a peptide, where a conserved tryptophan sandwiches the central valine of the peptide (Brasher *et al.,* 2000; Thiru *et al.,* 2004; Huang *et al.,* 2006). The affinity of the *Drosophila* HP1 chromo shadow domain for protein partners was compared (Mendez *et al.,* 2011). In addition to the PxVxL motif, a novel LCVKI was discovered (Mendez *et al.,* 2011). Affinity tightened by 50-fold, suggesting LCVKI-binding drives homodimerization of the HP1 protein. A novel function was attributed to the C-terminal extension (CTE). CTE is essential for stability of dimers and discriminatory binding of HP1 to a partner (Mendez *et al.,* 2011). Therefore, HP1 variants also target chromatin by relying on unique CTE regions.

Chromodomains of signal recognition particle in chloroplast

Plants rely on chloroplast signal recognition particles cpSRP54 and cpSRP43 for photosynthesis (Schuenemann *et al.,* 1998). The cpSRP43 protein contains three chromodomains with similar architecture (Sivaraja *et al.,* 2005); none forms the aromatic cage (Figure 4d). The first chromodomain is important for integrating light-harvesting chlorophyll-binding proteins (LHCPs) into thylakoids (Goforth *et al.,* 2004). The C-terminal α-helix of the second chromodomain in cpSRP43 facilitates binding to cpSRP54 with nanomolar affinity (Hermkes *et al.,* 2006; Sivaraja *et al.,* 2005). The second and third chromodomains interact with the Alb3 translocase protein, which is necessary for LHCP insertion into the thylakoid membrane (Falk *et al.,* 2010; Lewis *et al.,* 2010). We note that cpSRP43 protein is the only chromodomain protein with function outside of the nuclear boundary.

Chromodomain of ABC-type ATPase

Elongation factor eEF3 belongs to a family of ATP-binding cassette (ABC) proteins that functions in transporting proteins across membranes, DNA repair and translation (Andersen *et al.,* 2006). Interestingly, in yeast, this protein interacts with ribosomes for efficient tRNA release. It is speculated the chromodomain of this protein plays a role in this process by stabilizing an open ribosomal conformation. A chromodomain is not predicted, but the structural characterization of eEF3 revealed a chromodomain-like region. We performed a superposition of this region with the canonical chromodomain and identified extensive insertions in this domain (Figure 4e) (Andersen *et al.,* 2006). By contrast, SMART and InterPro predict the presence of an embedded chromodomain in a few other AAA helicase modules such as that in the [Nu+] prion formation protein 1 of yeast (Figure 1a). Therefore, bona fide chromodomains may also contribute to accessing substrates for other ABC proteins.

Distinctions of chromodomain from tudor clan modules

The chromodomain was originally noted to form a similar architecture to a small chromosomal protein in archaea called Sac7d (Ball *et al.,* 1997b). Sac7d forms a module that binds the minor groove of any DNA sequence at micromolar affinity to bend DNA for increased thermal stability (Robinson *et al.,* 1998). Additional work showed chromodomains form consistently smaller distinct modules with structural homology to a group of other eukaryotic domains collectively called the tudor clan. A hallmark of the chromodomains is the presence of a 21-residue chromobox homology motif (Figure 5a), originally noted

when CHD double chromodomains were characterized (Flanagan *et al.,* 2007). This region forms a highly superimposable unit of architecture among chromodomains and participates in partner recognition. Sequence insertions or deletions within this region rarely occur except in cpSRP43 chromoboxes (Figure 4d). At position 5 of a chromobox, there is always an aromatic residue. Other aromatic residues at positions 8 and/or 12 are required for methyllysine binding capability of chromodomains; the position 12 aromatic residue is only present in the MRG class (Figure 5a).

Despite low overall sequence identity between MBT, PWWP, Tudor, and Agenet domains, they superimpose well on the canonical chromobox (Figure 5). There is variability in inter-strand contacts, the folding of the turn and conservation of key aromatic residues associated with these other motifs. Tudor clan modules were coined a "Royal family" (Maurer-Stroh *et al.,* 2003), because they are often shown to be capable of methyllysine recognition by assembling related cages near the chromobox homology motif (Guo *et al.,* 2009; Vezzoli *et al.,* 2010; Min *et al.,* 2007; Botuyan *et al.,* 2006; Ramos *et al.,* 2006; Maurer-Stroh *et al.,* 2003). As compared to chromodomains, there are far less MBT, PWWP and Tudor domains in metazoan genomes, and the Agenet domain is a hallmark of plant genomes (Maurer-Stroh *et al.,* 2003).

Among the members of the tudor clan, the MBT domain forms a chromobox closest to that of chromodomains in the MRG class (Figure 5a and b). MBT domains are shown to be selective for monomethylated lysine, e.g., the human L3MBTL2 protein recognizes the H4K20 methyl-mark using a canonical chromobox related to the MSL3 chromodomain (Guo *et al.,* 2009). The 53BP1 tudor domains also bind the H4K20 methyl-mark using aromatic residues in positions 5 and 12 (Figure 5a and c) (Botuyan *et al.,* 2006). Lack of an aromatic residue at position 8 is also seen for the Agenet motif in the FMRP protein (Figure 5a and c), which interacts with several trimethylated lysine marks (Ramos *et al.,* 2006). For the PWWP motif of BRFP1, the aromatic residue at position 5 cooperates with residues outside the chromobox to interact with H3K36 methyl-mark, and aromatic residue at position 12 is not used (Figure 5d) (Vezzoli *et al.,* 2010). Therefore, the Tudor,

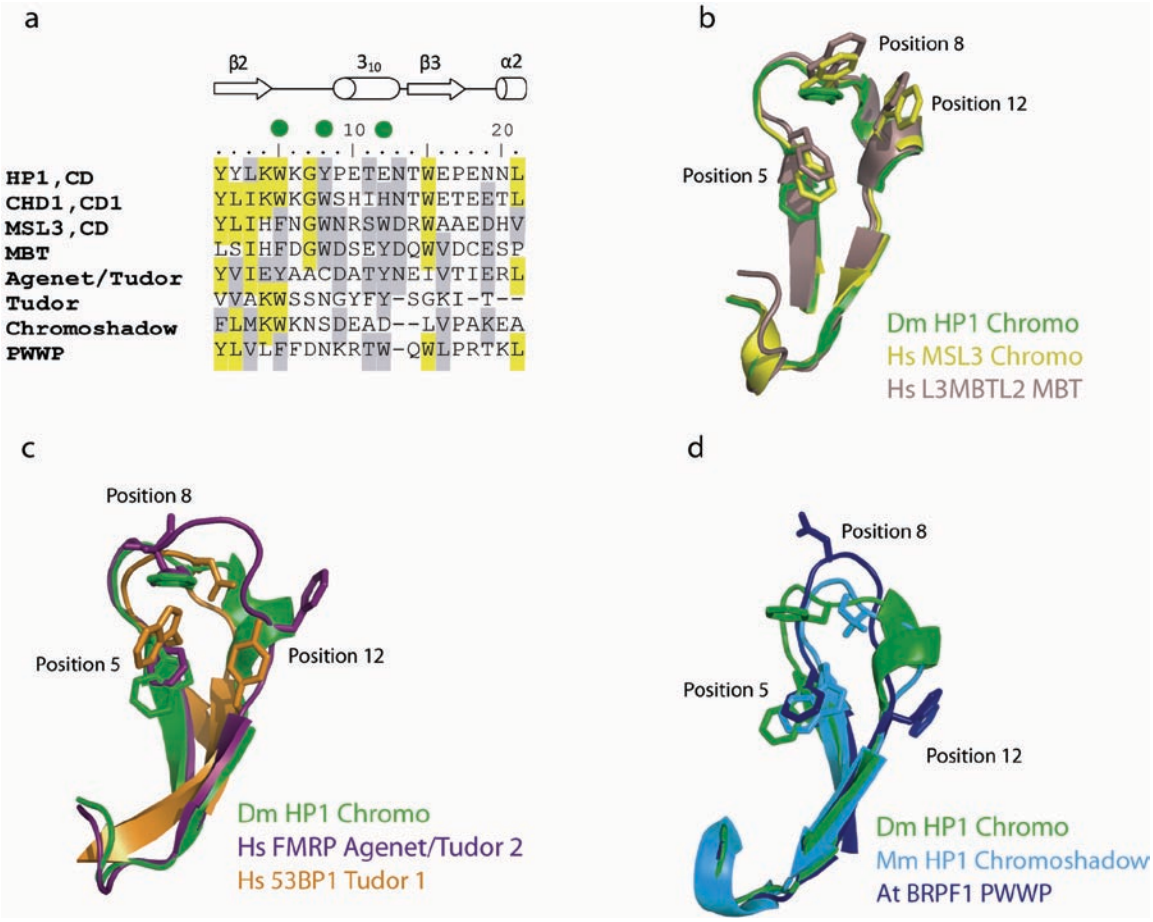

Figure 5. The chromobox homology motif is a toolkit for distinguishing the tudor clan modules from the chromodomain. (a) A sequence alignment of the chromobox from a selection of chromodomains and related domains: chromo shadow domain, tudor, MBT, PWWP and Agenet. (b–d) Ribbon diagram show superposition of the relevant regions of homology and point to key aromatic residues. The superposition of the *Drosophila* HP1 chromobox with related regions from other domains produced the following RMSD over the backbone residues: 1.21 Å with MBT, 0.44 Å with MSL3, 1.5 Å with 53BP1 Tudor, 2.7 Å with FRMP Agenet, 0.96 Å with chromo shadow and 1.45 Å with PWWP.

Agenet and PWWP modules each contain a functionally related motif that can be distinguished from chromodomains (Figure 5).

Dimethylarginine recognition by Tudor domains

The Tudor domain is also associated with recognition of methylated arginine, symmetrically-dimethylated (sDMA) or asymmetrically-dimethylated (aDMA). Their interactions with such glycine-rich peptides are closely related to the representative SMN tudor important for splicing. They exhibit millimolar affinity for binding methylated peptides of a variety of partner proteins. Recently, a surprising micromolar affinity interaction was described for a tudor domain binding to a sDMA partner peptide, and the structure of the complex suggested an asparagine-gated aromatic cage (Liu *et al.,* 2010). In the same protein, *Drosophila* Tudor, there are 10 other tudor domains, but they lack structural elements for methylated arginine recognition at high affinity.

With regard to the recognition of histone arginine methyl-marks, the mammalian TDRD3 tudor domain exhibits specificity for aDMA in histone H3 and H4 tails (Yang *et al.,* 2010). The same Tudor domain is also thought to be the effector for a novel aDMA found in the carboxy terminal domain of RNA Polymerase II (Sims *et al.,* 2011). A protein microarray screen had originally identified TDRD3 as an effector for sDMA peptides (Kim *et al.,* 2006). To what degree TDRD3 discriminates between sDMA and aDMA containing peptides has not been reported. To address this question, we prepared the construct used in protein microarray screen (Kim *et al.,* 2006), and used it in fluorescence polarization peptide binding assays as described (Jacobs *et al.,* 2004). We found TDRD3 binds to aDMA of H3R17 peptide with 50 μM affinity, whereas the binding to an sDMA of H3R17 exhibited a factor of 7 weaker affinities. For a structural basis for this selectivity, we inspected the solution structure deposited in the protein data bank for the human TDRD3 (pdb accession code 2D9T), and identified an unconserved tryptophan that buttresses TDRD3 aromatic cage, potentially enabling selectivity through van der Waals and π-cation interactions with the aDMA.

By contrast to the tudor domain, the chromodomain has never been noted for methylarginine recognition. This is likely due to the high conservation in the chromobox homology motif as well as key aromatic residues. These prohibit the insertion of a methyl arginine moiety in chromodomain aromatic cages. Therefore, the tudor domain sequences have evolved significantly to allow methyllysine or methylarginine selectivity. Interestingly, no chromodomain has been identified to recognize the methylated lysine in the core of histone H3. However, a protein microarray screen has identified a putative interaction between the tudor domain of C20orf104 (also called human PHF20) and the H3K79 methyl-mark (Kim *et al.,* 2006). Subsequent studies are needed to verify the interaction and identify the basis for the unique recognition of this methyl-mark.

Perspective

In less than a decade, enormous literature has been published on versatile functions of chromodomains in epigenetic regulations (a selection listed in Table 2). These have often described individual chromodomain modules, and the *in vitro* targeting potential for histone and other disordered peptides. However, NMR studies that characterized hydrogen exchange properties of the H3 tail in nucleosome arrays revealed a majority of the amide protons were protected from exchange, suggestive of a folded H3 tail (Kato *et al.,* 2009). The methylation of key residues in the H3 tail may thus increase its dynamics, and promote accessibility of individual nucleosomes in diverse gene expression mechanisms. Investigating the interaction of chromodomains with higher order chromatin structure was hampered by challenges in the reconstitution of an appropriate chromatin environment *in vitro*. However, recent efforts have verified physiologically relevant chromatin fibers can be reconstituted (Li *et al.,* 2010a). A recombinant method for preparation of histones harboring methyllysine analogs has also been validated for the study of HP1 chromodomain interaction with the H3K9 methyl-mark (Simon *et al.,* 2007).

Recently, a nucleosome-bridging mechanism for the HP1 adaptor protein was described (Canzio *et al.,* 2011). Binding studies between Swi6 and the H3K9-methylated nucleosome arrays led to chromodomain dimerization, and featured array organization related to the spreading of heterochromatin (Canzio *et al.,* 2011). These studies need further development to incorporate the crucial roles of the chromo shadow domain, the hinge and CTE regions (Mendez *et al.,* 2011; Smothers and Henikoff, 2001), which are required for heterochromatin assembly. Therefore, HP1 proteins stabilize a network of modification-dependent interactions. MSL3 chromodomain was also proposed to function in bridging of nucleosomes. The crystal structure of the complex of MSL3 chromodomain with DNA and the H4K20me1 peptide superimposes closely on the tetranucleosome structure at the junction of two nucleosomes (Kim *et al.,* 2010; Schalch *et al.,* 2005). As H4K16 acetylation disrupts MSL3 binding, the chromodomain was suggested to guide the spreading of the MSL complex to nucleosomes lacking acetylation.

Thus far, functions attributed to the majority of chromodomains have been linked to histones and nucleosome regulation. However, a wider usage of lysine methyltransferase activity has been noted for chromatin signaling pathways (Huang and Berger, 2008). The presence of lysine-methylated motifs in non-histone proteins suggests further research on chromodomains will broaden their role in mediating a variety of modification-dependent signaling networks (Table 2).

Declaration of interest

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