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JB Review

Biochemical and structural properties of heterochromatin protein 1: understanding its role in chromatin assembly

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Heterochromatin protein 1 (HP1) is an evolutionarily conserved chromosomal protein that binds lysine 9-methylated histone H3 (H3K9me), a hallmark of heterochromatin, and plays a crucial role in forming higher-order chromatin structures. HP1 has an N-terminal chromodomain and a C-terminal chromo shadow domain, linked by an unstructured hinge region. Although biochemical and structural studies have revealed each domain's properties, little is known about the mechanisms by which these domains cooperate to carry out HP1's function in forming higher-order chromatin structures. In this review, we summarize HP1's biochemical and structural properties and highlight the latest findings regarding HP1's interactions with nucleosomes.

Keywords: chromodomain/chromo shadow domain/heterochromatin/HP1/phosphorylation.

Abbreviations: CD, chromodomain; CK2, casein kinase II; CSD, chromo shadow domain; EMSAs, electrophoretic mobility-shift assays; HP1, heterochromatin protein 1; HP2, heterochromatin protein 2; NMR, nuclear magnetic resonance; Pc, Polycomb.

In eukaryotic cells, the organization of chromatin into higher-order structures plays an important role in a wide range of chromosomal processes. Heterochromatin, one of the most distinctive chromatin structures in the nucleus, is involved in such diverse cellular functions as nuclear organization, chromosome segregation, dosage compensation and epigenetic gene regulation. Heterochromatin is generally transcriptionally silent, and this higher-order chromatin structure can spread, causing the heritable inactivation of euchromatic genes adjacent to the heterochromatin and leading to variegated patterns of gene expression (*1–3*).

Heterochromatin protein 1 (HP1) is a non-histone chromosomal protein that was initially discovered in *Drosophila* as an enriched factor at highly condensed, transcriptionally inert regions of polytene chromosomes (*4*, *5*). HP1 is evolutionarily conserved, and its homologues and isoforms have been identified in eukaryotic organisms, ranging from fission yeast to mammals, flies, and nematodes (*6–9*). In mammals, the three HP1 isoforms HP1α, HP1β and HP1γ (Fig. 1A) have different localization patterns within cells (*11*, *12*). Mice deficient for each HP1 isoform exhibit different phenotypes (*13–15*), indicating that the isoforms have distinct functions in forming higher-order chromatin structures.

HP1-family proteins share a basic structure consisting of an amino-terminal chromodomain (CD) and a carboxyl-terminal chromo shadow domain (CSD) linked by an unstructured hinge region (*16*). The CD functions as a binding module that targets lysine 9-methylated histone H3 (H3K9me) (*17*, *18*), whereas the CSD functions as a dimerization module that provides a recognition surface for binding other proteins (*19–21*). In contrast, the hinge region and the N- and C-terminal tails are less conserved, and are thought to be unstructured. Thus, these regions are assumed to contribute to isoform-specific functions. Although the contribution of HP1-family proteins in silent heterochromatin assembly is widely recognized, recent studies have revealed their roles in other biological processes, including the cell cycle, transcriptional activation, telomere maintenance and DNA repair (*16*, *22–24*). In addition, an H3K9me-independent association of HP1 with chromatin has been demonstrated in *Drosophila* genome-wide analysis (*25*).

Although recent studies have done much to reveal the diverse cellular functions of HP1-family proteins, the mechanical contribution of each HP1 domain in forming higher-order chromatin structures remains obscure. In this review, we outline the biochemical properties of each of HP1 domain, and discuss how HP1 integrates each domain's distinctive functions to form silent heterochromatin.

The CD: A Recognition Module for H3K9me

HP1's CD, first identified in a comparison with the amino acid sequence of *Drosophila* Polycomb (Pc) (*26*), was later confirmed to be a requirement for HP1's association with chromatin (*27*). After the landmark discovery of the histone methyltransferase activity of the SUV39H-family proteins (*28*), several studies demonstrated that the K9 tri-methylated histone H3 (H3K9me3) is a hallmark of heterochromatin, and that the CD is responsible for HP1's direct interaction with H3K9me3 (*29–31*).

The three-dimensional (3D) structure of the HP1 CD has been solved by nuclear magnetic resonance (NMR) spectrometry (*32*) and X-ray crystallography (*18*, *33*). The CD consists of four curved β-strands and

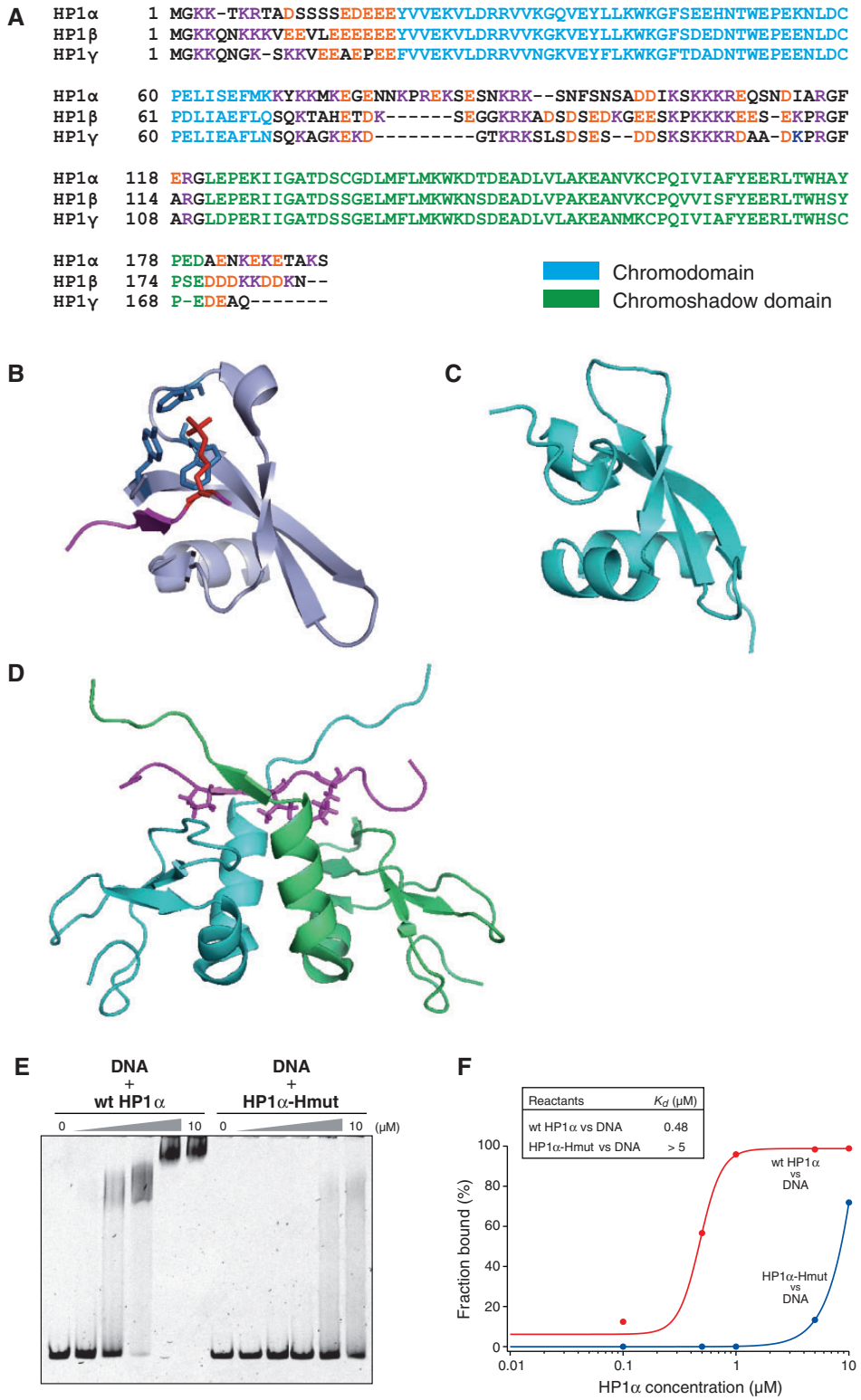

Fig. 1 HP1's biochemical and structural properties. (**A**) The amino acid sequences of human HP1α, HP1β and HP1γ, aligned by ClustalW software. Amino acids comprising the CD and CSD are shown in light blue and green, respectively. Acidic and basic amino acids are shown in orange and purple, respectively, in the hinge region and the N-terminal and C-terminal tails. (**B**) The structure of human HP1α CD (grey) in complex with lysine 9-methylated histone H3 peptide (red). Three conserved amino acids that form the aromatic cage are indicated. PDB code: 3FDT. (**C**, **D**) The structure of the mouse HP1β CSD monomer (C) and dimer (light blue and green) in complex with a peptide from chromatin assembly factor 1 (CAF1) subunit A (purple) (D). Amino acids corresponding to the PXVXL motif are shown. PDB code: 1S4Z. (**E**) EMSAs using wild-type (wt) and hinge-mutated (Hmut) HP1α. A 193-bp double-stranded 601 DNA (*10*) was used as a probe. HP1α-Hmut carries amino acids substitutions (K89A, R90A, K91A, K104A, K105A and K106A) in the hinge region. (**F**) The fraction of bound DNA probe, determined by EMSA (E), was plotted against the HP1α concentration. Dissociation constants measured by titration EMSAs are also shown (top, left).

an α-helix, and it recognizes H3K9me3 through an aromatic cage formed by three conserved residues (Fig. 1B). Most chromatin protein CDs form a similar 3D structure, and can recognize methylated histone H3 (*34*, *35*). Mutations in this domain or in the residues forming the cage disrupt the HP1 localization patterns, suggesting that the CD has a primary role in HP1's targeting of heterochromatin (*27*, *36*). Structurally, the HP1 CD is related to the Royal family of protein domains, which includes the Tudor, plant Agenet, Chromo, PWWP and malignant brain tumour domains (*37*). Since these domains fold similarly and recognize methylated histone tails, they are proposed to be descendants of an ancestral module that recognizes methylated ligands.

A series of *in vitro* biochemical analyses demonstrated that HP1 CD preferentially binds H3K9me3 over H3K9me2 or H3K9me1, and that each HP1 isoform's CD displays a different binding affinity for H3K9me3 (*38–40*). For example, the HP1β CD shows the highest binding affinity for the H3K9me3 peptide, which is four to six times stronger than that of the HP1α CD's affinity (*38*, *39*). Notably, however, the interaction of any CD with the H3K9me3 peptide is rather weak, with only a micromolar-order dissociation constant Kd (*18*, *33*, *38*, *39*). Since HP1-family proteins form stable dimers, it appears that two CDs may cooperate to increase their overall affinity for the H3K9me3 nucleosome. Alternatively, other HP1 domain(s) may participate in recognizing and/or binding the H3K9me3-enriched chromatin (*16*).

The HP1-family proteins possess a stretch of acidic amino acids right next to the CD (Fig. 1A), but the functional role of this acidic region remains unclear (*27*, *41*). Mammalian HP1α contains serine residues just upstream of this acidic region (Fig. 1A, S11-14), the phosphorylation of which enhances the HP1α CD's binding to the H3K9me3 peptide (*39*). Moreover, phosphorylation-deficient HP1α fails to localize to pericentromeric heterochromatin. These results support the idea that HP1α's N-terminal phosphorylation is functionally linked with the adjacent acidic residues, and contributes to the HP1α CD's binding of H3K9me3 (*39*). Of note, *Drosophila* HP1a is also phosphorylated at serine 15, which is in a similar position to the serines phosphorylated in HP1α, and mutation of the serine residue causes HP1a delocalization (*42*), suggesting an evolutionarily conserved mechanism to assist the H3K9me3-recognizing module. Since the histone H3 tail is highly basic, it is likely that the N-terminal acidic residues and adjacent phosphorylated serine(s) cooperate to increase HP1's binding affinity for the H3 tail via electrostatic interactions (Fig. 2A) (*39*).

The CD's intrinsic DNA-binding activity may provide another means of increasing HP1's affinity for the H3K9me3 nucleosome. Although the CD's DNA- or RNA-binding activity has been demonstrated for other proteins, such as fission yeast *Schizosaccharomyces pombe* Chp1 and mammalian Cbx proteins (*50–52*), the surface of the HP1 CD is negatively charged (*53*); this charge is likely to drive interaction with positively charged histone tails, but not with negatively charged DNA. Indeed, CDs of the HP1-family proteins have not been shown to bind DNA.

The CSD: A Module for Dimer Formation

The carboxyl-terminal CSD was first identified by its sequence similarity to the CD (*54*). As predicted, the overall structure of the CD and CSD is similar (Fig. 1C). However, CSD forms a symmetrical dimer and provides a hydrophobic pocket at the dimer interface that mediates interactions with other proteins (Fig. 1D) (*19*, *21*, *55*). Many of CSD's interaction partners contain a PXVXL pentapeptide motif, which was originally identified in a phage display study of fly HP1a (*56*), and structural studies revealed that the PXVXL-containing peptide interacts with the hydrophobic pocket formed by the β-sheets of CSD dimers (Fig. 1D) (*21*).

Although the PXVXL motif has been identified in a number of proteins that interact with HP1 in mammals and flies, a structural analysis of the residues that make contact with the PXVXL motif predicted that the CSDs in other organisms have altered specificities for interacting with other proteins (*21*). In addition, detailed analysis of the interaction between *Drosophila* HP1a and heterochromatin protein 2 (HP2), a non-histone chromosomal protein that associates with HP1a, identified a LCVKI motif in HP2 that binds HP1a's CSD (*57*). The binding affinity of the HP2 fragment is approximately twice than that of a peptide containing PXVXV, which is a PXVXL variant motif found in PIWI proteins. Although a central valine surrounded by bulky, hydrophobic residues in the +2/−2 positions appears to be a common feature of these motifs, other motifs that do not fit this consensus may mediate interactions with HP1's CSD dimer. It is also possible that different combinations of CSD dimers display unique specificities, as the three mammalian HP1 isoforms form various heterodimers (*12*). Although the dimerized CSD is the module best known for interacting with other proteins, a group of proteins containing a C2H2 zinc finger-type motif can interact with a dimerization-deficient mutant CSD (*58*).

Hinge Region: More than a Mere Connector

The hinge region connecting the CD and CSD is less evolutionarily conserved than these domains, and is structurally disordered (Fig. 1A) (*6*, *59*). Despite being less conserved, several studies of HP1 in different organisms have demonstrated that the hinge region binds DNA and RNA without any particular sequence specificity, and that several stretches of basic residues in the hinge region contribute to this binding (Fig. 1E and F) (*44*, *45*, *60–63*).

Given the weak interaction between the HP1 CD and H3K9me3, it is likely that DNA/RNA binding involving the hinge region increases HP1's binding affinity for the H3K9me3-containing nucleosome to promote its chromatin binding. Several studies show that HP1 localization and nuclear RNA are closely linked. RNase treatment abolishes HP1's heterochromatic localization in mouse cells permeabilized with Triton

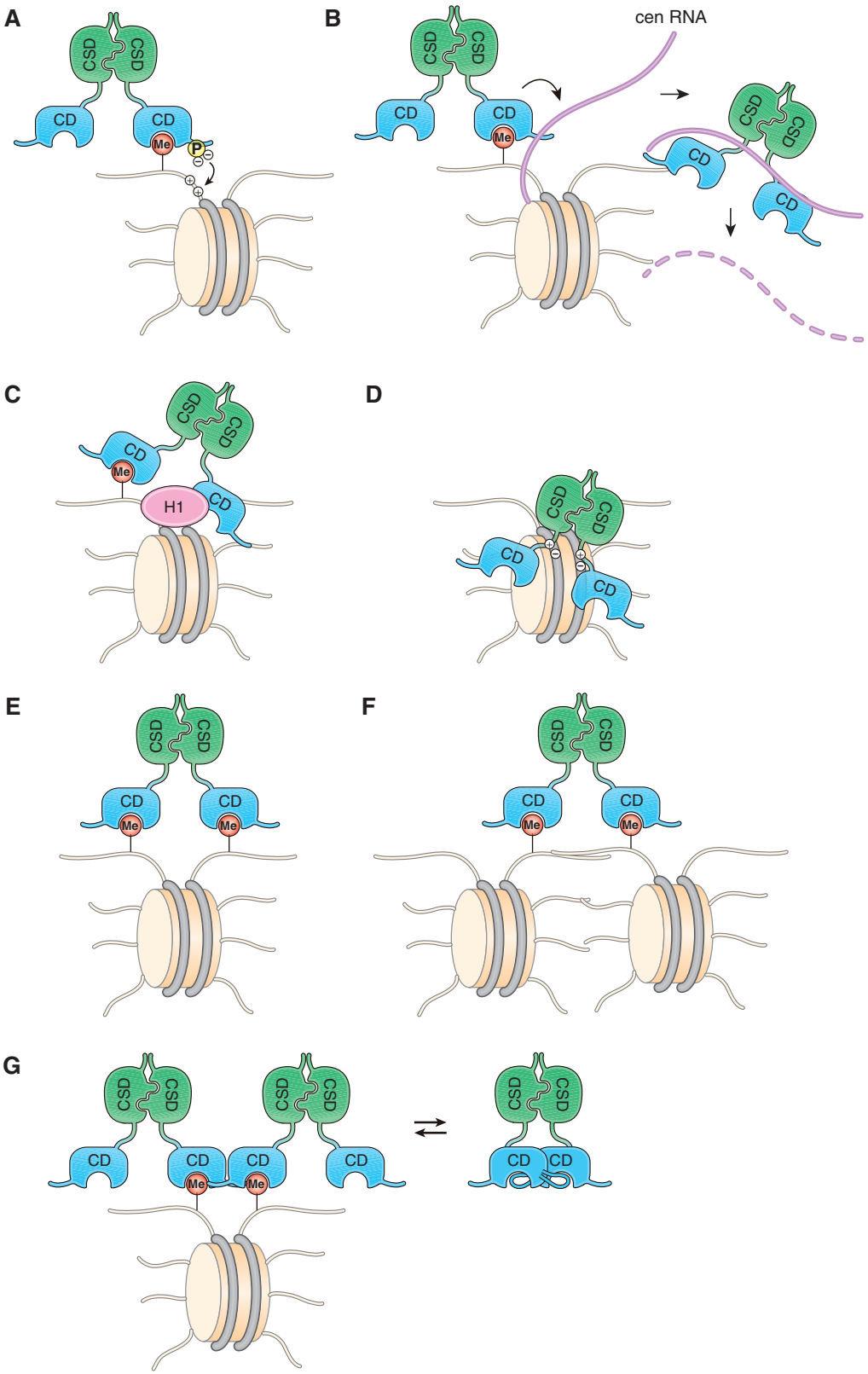

Fig. 2 Models for HP1's interactions with nucleosomes. HP1's N-terminal phosphorylation enhances its interaction with H3K9me3 (*39*, *42*, *43*). (**B**) HP1 binds newly synthesized RNA, and dissociates from H3K9-methylated nucleosomes to sequester and degrade the RNA (*44*). (**C**) HP1 preferentially binds nucleosomes containing linker histone H1 (*45*). (**D**) In the absence of H3K9me3, HP1 weakly interacts with the nucleosome via DNA-binding activity associated with the hinge region (*46*). (**E**, **F**) A HP1 dimer bridging two H3K9me3 tails on the same (E) or neighbouring nucleosomes (F) (*47*). (**G**) HP1 binds a mononucleosome as a tetramer through interactions between two CDs (left) (*48*). CD autoinhibits the free HP1 dimer (*49*).

Table I. Post-translational modifications of HP1.

Species	Isoform	Residue	PTMs	Enzyme	Function	Mutant defect	Reference
Human	β	T51	Phos	CK2	Dissociation from damaged chromatin	T51A,E > delocalization	Ayoub Ayoub *et al.* (*22*)
	γ	S83	Phos	PKA	Interaction with Ku70 Euchromatic localization	S83D > silencing defect	Lomberk *et al.* (*70*)
	γ	S83	Phos	Aurora A	Mitotic spindle localization	S83D,A > cell proliferation failure	Grzenda *et al.* (*72*)
Mouse	α	S11-14	Phos	CK2	Promotion of H3K9me binding	S11-14A > defect in H3K9me binding S11-14A > chromosomal instability	Hiragami-Hamada *et al.* (*39*)
	α	S93	Phos	N/D	N/D	N/D	Hiragami-Hamada *et al.* (*39*)
	α	*K*s in the hinge region	SUMO	UBC9	Interaction with non-coding RNA	N/D	Maison *et al.* (*71*)
	β	N/D	SUMO	N/D	Interaction with noncoding RNA	N/D	Maison *et al.* (*71*)
	γ	N/D	SUMO	N/D	Interaction with noncoding RNA	N/D	Maison *et al.* (*71*)
Drosophila	a	S15,S202	Phos	CK2	Heterochromatic localization	S15A,E, S202A,E > silencing defect	Zhao *et al.* (*42*)
S. pombe	Swi6	Ss in the N-terminus	Phos	CK2	Recruitment of HDAC complex	multiple S→A > silencing defect	Shimada *et al.* (*73*)
		K103	SUMO	Hus5/ Ubc9	Heterochromatin assembly	K103R > silencing defect	Shin *et al.* (*74*)
	Chp2	K198	SUMO	Hus5/ Ubc9	Heterochromatin assembly	K198R > silencing defect	Shin *et al.* (*74*)

N/D means not determined.

X-100 (*64*). An *in vitro* overlay assay using paraformaldehyde-fixed NIH3T3 cells also showed that HP1's heterochromatic localization is sensitive to RNase treatment, and requires both the CD and hinge regions (*45*). These results indicate that nuclear RNAs somehow guide HP1 to the target heterochromatin. A recent study showed that Swi6, an *S. pombe* HP1 homologue, also binds RNA, and that RNA and H3K9me3 compete to bind Swi6 (*44*). From this competitive binding, Keller *et al.* proposed a model in which HP1 binds newly synthesized RNA, and then sequesters and degrades it by dissociating from the H3K9 methylated nucleosome (Fig. 2B).

As described above, nuclear RNAs are likely to become physiological targets of HP1, and several studies using *in vitro* electrophoretic mobility-shift assays (EMSAs) suggested that HP1 has a higher affinity for RNA than for DNA (*44*, *45*). However, other studies implicate the hinge region in HP1's nucleosome binding (*45*, *61*, *63*). Of particular interest, *Xenopus* HP1α preferentially binds to nucleosomes with linker histones, and the hinge region is involved in this preference for linker histone-bound nucleosomes (*45*). Therefore, DNA-binding activity associated with the hinge region may stabilize interactions with a subset of nucleosomes associated with linker histones (Fig. 2C). It remains to be determined whether HP1's physiological target is the nucleosomal DNA, the transcribed RNAs or both.

N-Terminal and C-Terminal Tails

Although CD and CSD are highly conserved among HP1-family proteins across different organisms, the hinge and the N- and C-terminal tails are less evolutionarily conserved and are thought to be unstructured (*6*). Even so, a stretch of acidic residues is found right next to the CD in almost all HP1 proteins (Fig. 1A), and there are clusters of basic residues upstream of the acidic stretch. Although this clustered distribution of basic and acidic amino acid residues is observed in *Drosophila* HP1a, *S. pombe* Swi6, and mammalian and *Xenopus* HP1 isoforms, this characteristic is not clearly observed in other HP1 isoforms (*65–67*). The presence of these charged amino acid clusters may contribute to isoform-specific function in each organism.

As described above, the stretch of acidic amino acids and phosphorylated serine residues in mammalian HP1α appears to promote interactions between the CD and the H3 tail (Fig. 2A) (*39*). Although the functional implications of the N-terminal patch of basic residues remain unclear, NMR analysis revealed that these residues are involved in HP1β's interaction with unmodified nucleosomes and with free DNA (*46*). It is therefore conceivable that the N-terminal basic residues cooperate with the hinge region to interact with nucleosomal DNA (Fig. 2D). In addition, the distribution of alternating acidic and basic clusters in the N-terminal and hinge regions (Fig. 1A) may serve to loosely fix the relative position of HP1 to the nucleosome.

The C-terminal tail extending from the CSD is short, with no known functional role, although it is enriched in charged amino acid residues in the mammalian HP1 isoforms (Fig. 1A) (*21*). Interestingly, recent structural and functional analyses of the *Drosophila* HP1a CSD revealed that the C-terminal tail forms an ordered structure when HP2 peptide binds the CSD and

modulates the binding strength (*57*, *68*). Accordingly, it is possible that C-terminal extensions assist in determining the CSD target specificity and/or binding strength in other HP1-family proteins as well.

HP1 Post-translational Modifications

There is growing evidence that HP1-family proteins can undergo a variety of post-translational modifications (*69*) (Table I). Mammalian HP1 isoforms are modified in a manner analogous to histones, including phosphorylation, acetylation, methylation, ubiquitination and SUMOylation (*69–71*). Of these, phosphorylation has been the most intensively studied. A metabolic labelling study revealed that while human HP1α and HP1γ are phosphorylated throughout the cell cycle and acquire additional phosphorylation(s) during the mitotic phase, HP1β appears to remain unphosphorylated (*11*). Detailed biochemical analyses showed that HP1α is multiply phosphorylated at its N-terminal serine residues (S11-14), and that this phosphorylation enhances HP1α's affinity for the H3K9me3 peptide (Fig. 2A) (*39*). Although mitotic phosphorylation has been mapped to the hinge region, its functional implication remains to be determined.

In *Drosophila*, an N-terminal serine (S15) of HP1a is phosphorylated by casein kinase II (CK2), and this phosphorylation plays a role in heterochromatin-mediated silencing (*42*, *43*). Given S15's relative position, it is likely that its phosphorylation increases *Drosophila* HP1a's affinity for H3K9me3, as is similarly observed for mouse HP1α (*39*). Thus, the phosphorylation-mediated regulation of CD function appears to be evolutionarily conserved. In *S. pombe*, Swi6 is phosphorylated by CK2, and this phosphorylation is required to recruit the histone deacetylase complex to chromatin (*73*). Although the method by which Swi6's phosphorylation modulates the recruitment of *trans*-acting factors remains unresolved, phosphorylation sites have been mapped to the Swi6 N-terminal region, and thus it is possible that phosphorylation alters Swi6's chromatin-binding activity, thereby affecting the recruitment of *trans*-acting factors.

Several studies have demonstrated that phosphorylation plays diverse roles in HP1's function. For instance, HP1γ phosphorylation on Ser-83 mediates HP1γ's interactions with Ku70, impairs its silencing activity and controls cell cycle progression (*70*, *72*). In addition, HP1β phosphorylation on Thr-51 has been linked to the HP1β mobilization after DNA damage (*22*). The HP1β Thr-51 lies within the CD, and its phosphorylated state appears to diminish H3K9me3 binding. Although Thr-51 and its surrounding amino acids are highly conserved among the three mammalian HP1 isoforms, we were unable to obtain direct evidence that CK2 phosphorylates HP1α's Thr-51 *in vitro* (our unpublished observation). Structural differences between HP1α CD and HP1β CD might affect their accessibility to CK2, or other cellular factor(s) may be required for efficient phosphorylation by CK2.

Drosophila HP1a is phosphorylated at serine 202 (Ser 202) in the C-terminal tail, and mutation analysis suggested that this phosphorylation is critical for heterochromatin assembly (*42*, *43*). A recent structural study indicated that mutations mimicking the phosphorylation at Ser 202 increase the binding affinity between CSD and HP2 peptide (*57*). These studies further support the idea that phosphorylation modulates HP1's interactions with other proteins. In this respect, HP1a's C-terminal phosphorylation may be functionally linked with the acidic residues in the C-terminal regions of mammalian HP1 isoforms.

Among other post-translational modifications, functional interactions between SUMOylation and HP1 have been demonstrated in mouse and *S. pombe* cells (*71*, *74*). In mouse cells, HP1α is SUMOylated at the hinge region, and the SUMOylated HP1α interacts with non-coding RNAs corresponding to major satellite repeats (*71*). *De novo* localization assays showed that SUMOylation is critical for the initial targeting of HP1α. *In vitro* SUMOylation assays also showed that more than one lysine residue in the hinge region can be SUMOylated. Although how the SUMO modification promotes HP1α's RNA binding is unresolved, the SUMO modification might loosely alter the unstructured hinge region favourably for RNA binding. In *S. pombe*, the HP1 homologues Swi6 and Chp2 are SUMOylated at their conserved lysine residues in the CD, and cells expressing SUMO-deficient mutant Swi6 or Chp2 show silencing defects (*74*). Although it is not clear how SUMO modification contributes to Swi6 and Chp2 functions, findings in mouse cells indicate that the SUMO moiety may promote HP1's interaction with non-coding RNAs transcribed from heterochromatin.

HP1 Dynamics and Histone Modifications

In contrast to the cytological appearance of heterochromatin, the interaction between HP1 and chromatin is not static, but rather is highly dynamic (*36*, *75*). Fluorescence recovery after photobleaching showed that HP1 is highly mobile in mammalian living cells, even within the heterochromatin domains. Further study using *S. pombe* showed that Swi6 interacts dynamically with heterochromatin (*76*). Notably, this study indicated the existence of at least two kinetically distinct populations of Swi6 in heterochromatin. From these cytological studies, it appears that a population of HP1 associates stably with H3K9me3-containing nucleosomes, whereas the remainder associates with the domain only weakly, presumably via a CSD-mediated interaction. Although the role of the latter HP1 population remains unclear, accumulated HP1 may form a domain that restricts the access of other *trans*-acting factors, including RNA polymerase II. The kinetics of these interactions can change according to differentiation and other cellular conditions (*75*, *77*). The underlying molecular mechanisms modulating HP1 dynamics during cellular differentiation remain elusive.

Although HP1 associates stably with heterochromatic domains during interphase, its localization

changes dramatically during mitosis (*78*). In human cells, HP1β and HP1γ almost completely disappear from mitotic chromosomes, whereas HP1α localizes weakly to the centromeres (*79*). Dynamic changes in HP1 localization are tightly correlated with the appearance of histone H3 Serine 10 phosphorylation (H3S10phos) (*38*, *78*, *80*). Although H3K9me3 is present throughout the cell cycle, HP1 CD can only weakly interact with dually modified H3 (H3K9me3S10phos). In addition, inhibiting Aurora B, a mitotic kinase, results in HP1's persistent association with mitotic chromosomes. These studies indicate that H3S10phos acts as a molecular switch to modulate interactions between HP1 and chromatin (*78*).

Although the relationship between HP1 dissociation and the acquisition of H3S10phos appears solid, several studies have argued against H3S10phos having a primary role. *In vitro* pull-down analyses demonstrated that full-length HP1α is able to interact with an H3-tail peptide dually modified with K9me3 and S10phos (*81–83*). Although interactions between H3K9me 3S10phos and HP1β or HP1γ have yet to be examined, HP1α's ability to interact with H3 with dual modifications may be linked with its localization on the mitotic chromosome (*79*). In addition to the CD, other HP1 domains may contribute to HP1α's interaction with H3K9me3S10phos. Alternatively, other post-translational modifications on HP1 may modulate changes in its dynamic localization during mitosis. In this respect, metaphase-specific HP1 phosphorylation appears to be a likely regulator of HP1 dynamics during mitosis (*11*, *39*).

Interactions between HP1 and Nucleosomes

Recently, several groups have examined interactions between recombinant HP1 and reconstituted nucleosomes (*46–49*, *63*). Using reconstituted nucleosomes with enzymatically introduced H3K9me3, Mishima *et al*. (*63*) demonstrated that human HP1α has a high binding affinity for H3K9me3 nucleosomes, and that charged amino acids both in the hinge region and the CSD contribute to HP1α's selective binding to the H3K9me3 nucleosome. Another group prepared H3K9me3 nucleosomes using a methyl lysine analogue (H3Kc9me3), and demonstrated that the H3K9me3 mark alters the interaction between HP1β and the nucleosome: the methyl mark promotes the dynamic interaction between HP1β's CD and the H3 tail, whereas in the absence of methylation, HP1β interacts with the nucleosome only weakly, via DNA-binding activity associated with the hinge and N-terminal regions (Fig. 2D) (*46*). Although the experimental systems varied, these studies indicated that DNA binding associated with the hinge region modulates HP1's binding with the H3K9me3 nucleosome.

One group reconstituted H3K9me3-containing nucleosome arrays, and demonstrated that HP1α preferentially interacts with arrays containing H3K9me3 when compared with control arrays lacking the H3K9me3 mark (*47*). By directly monitoring the nucleosome arrays by electron microscopy, this group also showed that HP1α promoted nucleosome associations within an array, leading to chromatin condensation. In this system, the hinge region appeared to contribute little to the nucleosome association, and the hinge region's DNA-binding activity, which mediates HP1's interaction with unmodified nucleosomes, was weakened under physiological ionic strength (*47*). Canzio *et al*. (*48*), using *S. pombe* Swi6 to examine HP1 interactions, demonstrated that Swi6's specificity for the H3K9me3 mark was lower in mononucleosomes, and increased in nucleosome arrays. As with human HP1, Swi6's association with unmodified mononucleosomes or multinucleosomes appears to involve the hinge region's DNA-binding activity. More work is necessary to determine the functional implications of the hinge region and its binding of DNA and unmodified nucleosomes.

Although HP1 forms a stable dimer, some experimental systems have suggested the presence of further multimeric interactions (*48*, *84*). From its structural features, it is apparent that the HP1 dimer is capable of bridging two H3K9me3 tails, whether on the same or neighbouring nucleosomes (Fig. 2E and F). Interestingly, using sedimentation-velocity analytical ultracentrifugation, Canzio *et al*. (*48*) showed that Swi6 binds a mononucleosome as a tetramer, and proposed that the interaction between two CDs promotes multimeric Swi6 interactions on the nucleosomes (Fig. 2G). The same group demonstrated that the Swi6 CD contains a loop mimicking the H3 tail, and that this loop plays an auto-inhibitory role for free Swi6 dimers (*49*). These models are attractive, given HP1's ability to bridge nucleosomes. However, a similar CD–CD interaction has yet to be identified in other HP1 proteins (*46*, *59*), and the H3 tail-mimicking loop has not been clearly identified in other HP1 CDs. Further studies are needed to clarify how CD–CD interactions contribute to heterochromatin assembly.

Concluding Remarks

Over the past two decades, extensive studies of HP1, a major structural component of heterochromatin, have increased our understanding of the biochemical properties of each HP1 domain. However, little is known about the mechanisms by which the individual domains cooperate to promote HP1's chromatin targeting and, more importantly, the assembly of higher-order chromatin structures. With recent technical advances, reconstituted nucleosomes can now be prepared with particular histone modifications to explore the interactions between HP1 and H3K9me3 nucleosomes. However, it is still unclear how HP1's interactions with nucleosomes are regulated by its phosphorylation and other post-translational modifications. Furthermore, how HP1's DNA-/RNA-binding activity is linked to chromatin targeting and assembly has yet to be determined. Further biochemical studies will provide significant insights into the function of HP1-family proteins in higher-order chromatin assembly.

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Conflict of Interest

None declared.

References

1. Grewal, S.I. and Elgin, S.C. (2002) Heterochromatin: new possibilities for the inheritance of structure. *Curr. Opin. Genet. Dev.* **12**, 178–187
2. Grewal, S.I. and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. *Science* **301**, 798–802
3. Richards, E.J. and Elgin, S.C. (2002) Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell* **108**, 489–500
4. James, T.C. and Elgin, S.C. (1986) Identification of a nonhistone chromosomal protein associated with heterochromatin in *Drosophila melanogaster* and its gene. *Mol. Cell. Biol.* **6**, 3862–3872
5. James, T.C., Eissenberg, J.C., Craig, C., Dietrich, V., Hobson, A., and Elgin, S.C. (1989) Distribution patterns of HP1, a heterochromatin-associated nonhistone chromosomal protein of *Drosophila*. *Eur. J. Cell Biol.* **50**, 170–180
6. Maison, C. and Almouzni, G. (2004) HP1 and the dynamics of heterochromatin maintenance. *Nat. Rev. Mol. Cell Biol.* **5**, 296–304
7. Lomberk, G., Wallrath, L., and Urrutia, R. (2006) The heterochromatin protein 1 family. *Genome Biol.* **7**, 228
8. Zeng, W., Ball, A.R. Jr, and Yokomori, K. (2010) HP1: heterochromatin binding proteins working the genome. *Epigenetics* **5**, 287–292
9. Levine, M.T., McCoy, C., Vermaak, D., Lee, Y.C., Hiatt, M.A., Matsen, F.A., and Malik, H.S. (2012) Phylogenomic analysis reveals dynamic evolutionary history of the *Drosophila* heterochromatin protein 1 (HP1) gene family. *PLoS Genet.* **8**, e1002729
10. Arimura, Y., Tachiwana, H., Oda, T., Sato, M., and Kurumizaka, H. (2012) Structural analysis of the hexasome, lacking one histone H2A/H2B dimer from the conventional nucleosome. *Biochemistry* **51**, 3302–3309
11. Minc, E., Allory, Y., Worman, H.J., Courvalin, J.C., and Buendia, B. (1999) Localization and phosphorylation of HP1 proteins during the cell cycle in mammalian cells. *Chromosoma* **108**, 220–234
12. Nielsen, A.L., Oulad-Abdelghani, M., Ortiz, J.A., Remboutsika, E., Chambon, P., and Losson, R. (2001) Heterochromatin formation in mammalian cells: interaction between histones and HP1 proteins. *Mol. Cell* **7**, 729–739
13. Aucott, R., Bullwinkel, J., Yu, Y., Shi, W., Billur, M., Brown, J.P., Menzel, U., Kioussis, D., Wang, G., Reisert, I., Weimer, J., Pandita, R.K., Sharma, G.G., Pandita, T.K., Fundele, R., and Singh, P.B. (2008) HP1-beta is required for development of the cerebral neocortex and neuromuscular junctions. *J. Cell Biol.* **183**, 597–606
14. Brown, J.P., Bullwinkel, J., Baron-Luhr, B., Billur, M., Schneider, P., Winking, H., and Singh, P.B. (2010) HP1gamma function is required for male germ cell survival and spermatogenesis. *Epigenetics Chromatin* **3**, 9
15. Abe, K., Naruse, C., Kato, T., Nishiuchi, T., Saitou, M., and Asano, M. (2011) Loss of heterochromatin protein 1 gamma reduces the number of primordial germ cells via impaired cell cycle progression in mice. *Biol. Reprod.* **85**, 1013–1024
16. Hiragami, K. and Festenstein, R. (2005) Heterochromatin protein 1: a pervasive controlling influence. *Cell. Mol. Life Sci.* **62**, 2711–2726
17. Jacobs, S.A., Taverna, S.D., Zhang, Y., Briggs, S.D., Li, J., Eissenberg, J.C., Allis, C.D., and Khorasanizadeh, S. (2001) Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. *EMBO J.* **20**, 5232–5241
18. Nielsen, P.R., Nietlispach, D., Mott, H.R., Callaghan, J., Bannister, A., Kouzarides, T., Murzin, A.G., Murzina, N.V., and Laue, E.D. (2002) Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. *Nature* **416**, 103–107
19. Brasher, S.V., Smith, B.O., Fogh, R.H., Nietlispach, D., Thiru, A., Nielsen, P.R., Broadhurst, R.W., Ball, L.J., Murzina, N.V., and Laue, E.D. (2000) The structure of mouse HP1 suggests a unique mode of single peptide recognition by the shadow chromo domain dimer. *EMBO J.* **19**, 1587–1597
20. Cowieson, N.P., Partridge, J.F., Allshire, R.C., and McLaughlin, P.J. (2000) Dimerisation of a chromo shadow domain and distinctions from the chromodomain as revealed by structural analysis. *Curr. Biol.* **10**, 517–525
21. Thiru, A., Nietlispach, D., Mott, H.R., Okuwaki, M., Lyon, D., Nielsen, P.R., Hirshberg, M., Verreault, A., Murzina, N.V., and Laue, E.D. (2004) Structural basis of HP1/PXVXL motif peptide interactions and HP1 localisation to heterochromatin. *EMBO J.* **23**, 489–499
22. Ayoub, N., Jeyasekharan, A.D., Bernal, J.A., and Venkitaraman, A.R. (2008) HP1-beta mobilization promotes chromatin changes that initiate the DNA damage response. *Nature* **453**, 682–686
23. Goodarzi, A.A., Noon, A.T., Deckbar, D., Ziv, Y., Shiloh, Y., Lobrich, M., and Jeggo, P.A. (2008) ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. *Mol. Cell* **31**, 167–177
24. Liu, H., Galka, M., Mori, E., Liu, X., Lin, Y.F., Wei, R., Pittock, P., Voss, C., Dhami, G., Li, X., Miyaji, M., Lajoie, G., Chen, B., and Li, S.S. (2013) A method for systematic mapping of protein lysine methylation identifies functions for HP1beta in DNA damage response. *Mol. Cell* **50**, 723–735
25. Figueiredo, M.L., Philip, P., Stenberg, P., and Larsson, J. (2012) HP1a recruitment to promoters is independent of H3K9 methylation in *Drosophila melanogaster*. *PLoS Genet.* **8**, e1003061
26. Paro, R. and Hogness, D.S. (1991) The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. *Proc. Natl Acad. Sci. U. S. A.* **88**, 263–267
27. Platero, J.S., Hartnett, T., and Eissenberg, J.C. (1995) Functional analysis of the chromo domain of HP1. *EMBO J.* **14**, 3977–3986
28. Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z.W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C.P., Allis, C.D., and Jenuwein, T. (2000) Regulation

of chromatin structure by site-specific histone H3 methyltransferases. *Nature* **406**, 593–599

29. Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C., and Kouzarides, T. (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124
30. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., and Jenuwein, T. (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120
31. Nakayama, J., Rice, J.C., Strahl, B.D., Allis, C.D., and Grewal, S.I. (2001) Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* **292**, 110–113
32. Ball, L.J., Murzina, N.V., Broadhurst, R.W., Raine, A.R., Archer, S.J., Stott, F.J., Murzin, A.G., Singh, P.B., Domaille, P.J., and Laue, E.D. (1997) Structure of the chromatin binding (chromo) domain from mouse modifier protein 1. *EMBO J.* **16**, 2473–2481
33. Jacobs, S.A. and Khorasanizadeh, S. (2002) Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science* **295**, 2080–2083
34. Fischle, W., Wang, Y., Jacobs, S.A., Kim, Y., Allis, C.D., and Khorasanizadeh, S. (2003) Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev.* **17**, 1870–1881
35. Yap, K.L. and Zhou, M.M. (2011) Structure and mechanisms of lysine methylation recognition by the chromodomain in gene transcription. *Biochemistry* **50**, 1966–1980
36. Cheutin, T., McNairn, A.J., Jenuwein, T., Gilbert, D.M., Singh, P.B., and Misteli, T. (2003) Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science* **299**, 721–725
37. Maurer-Stroh, S., Dickens, N.J., Hughes-Davies, L., Kouzarides, T., Eisenhaber, F., and Ponting, C.P. (2003) The Tudor domain 'Royal Family': Tudor, plant Agenet, Chromo, PWWP and MBT domains. *Trends Biochem. Sci.* **28**, 69–74
38. Fischle, W., Tseng, B.S., Dormann, H.L., Ueberheide, B.M., Garcia, B.A., Shabanowitz, J., Hunt, D.F., Funabiki, H., and Allis, C.D. (2005) Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. *Nature* **438**, 1116–1122
39. Hiragami-Hamada, K., Shinmyozu, K., Hamada, D., Tatsu, Y., Uegaki, K., Fujiwara, S., and Nakayama, J. (2011) N-terminal phosphorylation of HP1α promotes its chromatin binding. *Mol. Cell. Biol.* **31**, 1186–1200
40. Sadaie, M., Kawaguchi, R., Ohtani, Y., Arisaka, F., Tanaka, K., Shirahige, K., and Nakayama, J. (2008) Balance between distinct HP1 family proteins controls heterochromatin assembly in fission yeast. *Mol. Cell. Biol.* **28**, 6973–6988
41. Wang, G., Ma, A., Chow, C.M., Horsley, D., Brown, N.R., Cowell, I.G., and Singh, P.B. (2000) Conservation of heterochromatin protein 1 function. *Mol. Cell. Biol.* **20**, 6970–6983
42. Zhao, T., Heyduk, T., and Eissenberg, J.C. (2001) Phosphorylation site mutations in heterochromatin protein 1 (HP1) reduce or eliminate silencing activity. *J Biol. Chem.* **276**, 9512–9518
43. Zhao, T. and Eissenberg, J.C. (1999) Phosphorylation of heterochromatin protein 1 by casein kinase II is required for efficient heterochromatin binding in *Drosophila*. *J Biol. Chem.* **274**, 15095–15100
44. Keller, C., Adaixo, R., Stunnenberg, R., Woolcock, K.J., Hiller, S., and Buhler, M. (2012) HP1(Swi6) mediates the recognition and destruction of heterochromatic RNA transcripts. *Mol. Cell* **47**, 215–227
45. Meehan, R.R., Kao, C.F., and Pennings, S. (2003) HP1 binding to native chromatin in vitro is determined by the hinge region and not by the chromodomain. *EMBO J.* **22**, 3164–3174
46. Munari, F., Soeroes, S., Zenn, H.M., Schomburg, A., Kost, N., Schroder, S., Klingberg, R., Rezaei-Ghaleh, N., Stutzer, A., Gelato, K.A., Walla, P.J., Becker, S., Schwarzer, D., Zimmermann, B., Fischle, W., and Zweckstetter, M. (2012) Methylation of lysine 9 in histone H3 directs alternative modes of highly dynamic interaction of heterochromatin protein hHP1beta with the nucleosome. *J. Biol. Chem.* **287**, 33756–33765
47. Azzaz, A.M., Vitalini, M.W., Thomas, A.S., Price, J., Blacketer, M.J., Cryderman, D.E., Zirbel, L.N., Woodcock, C.L., Elcock, A.H., Wallrath, L.L., and Shogren-Knaak, M.A. (2014) HP1Hsalpha promotes nucleosome associations that drive chromatin condensation. *J. Biol. Chem.* **289**, 6850–6861
48. Canzio, D., Chang, E.Y., Shankar, S., Kuchenbecker, K.M., Simon, M.D., Madhani, H.D., Narlikar, G.J., and Al-Sady, B. (2011) Chromodomain-mediated oligomerization of HP1 suggests a nucleosome-bridging mechanism for heterochromatin assembly. *Mol. Cell* **41**, 67–81
49. Canzio, D., Liao, M., Naber, N., Pate, E., Larson, A., Wu, S., Marina, D.B., Garcia, J.F., Madhani, H.D., Cooke, R., Schuck, P., Cheng, Y., and Narlikar, G.J. (2013) A conformational switch in HP1 releases auto-inhibition to drive heterochromatin assembly. *Nature* **496**, 377–381
50. Ishida, M., Shimojo, H., Hayashi, A., Kawaguchi, R., Ohtani, Y., Uegaki, K., Nishimura, Y., and Nakayama, J. (2012) Intrinsic nucleic acid-binding activity of Chp1 chromodomain is required for heterochromatic gene silencing. *Mol. Cell* **47**, 228–241
51. Yap, K.L., Li, S., Munoz-Cabello, A.M., Raguz, S., Zeng, L., Mujtaba, S., Gil, J., Walsh, M.J., and Zhou, M.M. (2010) Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol. Cell* **38**, 662–674
52. Bernstein, E., Duncan, E.M., Masui, O., Gil, J., Heard, E., and Allis, C.D. (2006) Mouse polycomb proteins bind differentially to methylated histone H3 and RNA and are enriched in facultative heterochromatin. *Mol. Cell. Biol.* **26**, 2560–2569
53. Kaustov, L., Ouyang, H., Amaya, M., Lemak, A., Nady, N., Duan, S., Wasney, G.A., Li, Z., Vedadi, M., Schapira, M., Min, J., and Arrowsmith, C.H. (2011) Recognition and specificity determinants of the human cbx chromodomains. *J. Biol. Chem.* **286**, 521–529
54. Aasland, R. and Stewart, A.F. (1995) The chromo shadow domain, a second chromo domain in heterochromatin-binding protein 1, HP1. *Nucleic Acids Res.* **23**, 3168–3173
55. Ye, Q., Callebaut, I., Pezhman, A., Courvalin, J.C., and Worman, H.J. (1997) Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR. *J. Biol. Chem.* **272**, 14983–14989
56. Smothers, J.F. and Henikoff, S. (2000) The HP1 chromo shadow domain binds a consensus peptide pentamer. *Curr. Biol.* **10**, 27–30

57. Mendez, D.L., Kim, D., Chruszcz, M., Stephens, G.E., Minor, W., Khorasanizadeh, S., and Elgin, S.C. (2011) The HP1a disordered C terminus and chromo shadow domain cooperate to select target peptide partners. *Chembiochem* **12**, 1084–1096
58. Nozawa, R.S., Nagao, K., Masuda, H.T., Iwasaki, O., Hirota, T., Nozaki, N., Kimura, H., and Obuse, C. (2010) Human POGZ modulates dissociation of HP1alpha from mitotic chromosome arms through Aurora B activation. *Nat. Cell Biol.* **12**, 719–727
59. Munari, F., Rezaei-Ghaleh, N., Xiang, S., Fischle, W., and Zweckstetter, M. (2013) Structural plasticity in human heterochromatin protein 1beta. *PLoS One* **8**, e60887
60. Sugimoto, K., Yamada, T., Muro, Y., and Himeno, M. (1996) Human homolog of *Drosophila* heterochromatin-associated protein 1 (HP1) is a DNA-binding protein which possesses a DNA-binding motif with weak similarity to that of human centromere protein C (CENP-C). *J. Biochem.* **120**, 153–159
61. Zhao, T., Heyduk, T., Allis, C.D., and Eissenberg, J.C. (2000) Heterochromatin protein 1 binds to nucleosomes and DNA in vitro. *J. Biol. Chem.* **275**, 28332–28338
62. Muchardt, C., Guilleme, M., Seeler, J.S., Trouche, D., Dejean, A., and Yaniv, M. (2002) Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1alpha. *EMBO Rep.* **3**, 975–981
63. Mishima, Y., Watanabe, M., Kawakami, T., Jayasinghe, C.D., Otani, J., Kikugawa, Y., Shirakawa, M., Kimura, H., Nishimura, O., Aimoto, S., Tajima, S., and Suetake, I. (2013) Hinge and chromoshadow of HP1alpha participate in recognition of K9 methylated histone H3 in nucleosomes. *J. Mol. Biol.* **425**, 54–70
64. Maison, C., Bailly, D., Peters, A.H., Quivy, J.P., Roche, D., Taddei, A., Lachner, M., Jenuwein, T., and Almouzni, G. (2002) Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. *Nat. Genet.* **30**, 329–334
65. Smothers, J.F. and Henikoff, S. (2001) The hinge and chromo shadow domain impart distinct targeting of HP1-like proteins. *Mol. Cell. Biol.* **21**, 2555–2569
66. Thon, G. and Verhein-Hansen, J. (2000) Four chromo-domain proteins of *Schizosaccharomyces pombe* differentially repress transcription at various chromosomal locations. *Genetics* **155**, 551–568
67. Couteau, F., Guerry, F., Muller, F., and Palladino, F. (2002) A heterochromatin protein 1 homologue in *Caenorhabditis elegans* acts in germline and vulval development. *EMBO Rep.* **3**, 235–241
68. Mendez, D.L., Mandt, R.E., and Elgin, S.C. (2013) Heterochromatin protein 1a (HP1a) partner specificity is determined by critical amino acids in the chromo shadow domain and C-terminal extension. *J. Biol. Chem.* **288**, 22315–22323
69. LeRoy, G., Weston, J.T., Zee, B.M., Young, N.L., Plazas-Mayorca, M.D., and Garcia, B.A. (2009) Heterochromatin protein 1 is extensively decorated with histone code-like post-translational modifications. *Mol. Cell. Proteomics* **8**, 2432–2442
70. Lomberk, G., Bensi, D., Fernandez-Zapico, M.E., and Urrutia, R. (2006) Evidence for the existence of an HP1-mediated subcode within the histone code. *Nat. Cell Biol.* **8**, 407–415
71. Maison, C., Bailly, D., Roche, D., Montes de Oca, R., Probst, A.V., Vassias, I., Dingli, F., Lombard, B., Loew, D., Quivy, J.P., and Almouzni, G. (2011) SUMOylation promotes de novo targeting of HP1alpha to pericentric heterochromatin. *Nat. Genet.* **43**, 220–227
72. Grzenda, A., Leonard, P., Seo, S., Mathison, A.J., Urrutia, G., Calvo, E., Iovanna, J., Urrutia, R., and Lomberk, G. (2013) Functional impact of Aurora A-mediated phosphorylation of HP1gamma at serine 83 during cell cycle progression. *Epigenetics Chromatin* **6**, 21
73. Shimada, A., Dohke, K., Sadaie, M., Shinmyozu, K., Nakayama, J., Urano, T., and Murakami, Y. (2009) Phosphorylation of Swi6/HP1 regulates transcriptional gene silencing at heterochromatin. *Genes Dev.* **23**, 18–23
74. Shin, J.A., Choi, E.S., Kim, H.S., Ho, J.C., Watts, F.Z., Park, S.D., and Jang, Y.K. (2005) SUMO modification is involved in the maintenance of heterochromatin stability in fission yeast. *Mol. Cell* **19**, 817–828
75. Festenstein, R., Pagakis, S.N., Hiragami, K., Lyon, D., Verreault, A., Sekkali, B., and Kioussis, D. (2003) Modulation of heterochromatin protein 1 dynamics in primary Mammalian cells. *Science* **299**, 719–721
76. Cheutin, T., Gorski, S.A., May, K.M., Singh, P.B., and Misteli, T. (2004) In vivo dynamics of Swi6 in yeast: evidence for a stochastic model of heterochromatin. *Mol. Cell. Biol.* **24**, 3157–3167
77. Meshorer, E., Yellajoshula, D., George, E., Scambler, P.J., Brown, D.T., and Misteli, T. (2006) Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Dev. Cell* **10**, 105–116
78. Dormann, H.L., Tseng, B.S., Allis, C.D., Funabiki, H., and Fischle, W. (2006) Dynamic regulation of effector protein binding to histone modifications: the biology of HP1 switching. *Cell Cycle* **5**, 2842–2851
79. Hayakawa, T., Haraguchi, T., Masumoto, H., and Hiraoka, Y. (2003) Cell cycle behavior of human HP1 subtypes: distinct molecular domains of HP1 are required for their centromeric localization during interphase and metaphase. *J. Cell Sci.* **116**, 3327–3338
80. Hirota, T., Lipp, J.J., Toh, B.H., and Peters, J.M. (2005) Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. *Nature* **438**, 1176–1180
81. Mateescu, B., England, P., Halgand, F., Yaniv, M., and Muchardt, C. (2004) Tethering of HP1 proteins to chromatin is relieved by phosphoacetylation of histone H3. *EMBO Rep.* **5**, 490–496
82. Terada, Y. (2006) Aurora-B/AIM-1 regulates the dynamic behavior of HP1alpha at the G2-M transition. *Mol. Biol. Cell* **17**, 3232–3241
83. Vermeulen, M., Eberl, H.C., Matarese, F., Marks, H., Denissov, S., Butter, F., Lee, K.K., Olsen, J.V., Hyman, A.A., Stunnenberg, H.G., and Mann, M. (2010) Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. *Cell* **142**, 967–980
84. Haldar, S., Saini, A., Nanda, J.S., Saini, S., and Singh, J. (2011) Role of Swi6/HP1 self-association-mediated recruitment of Clr4/Suv39 in establishment and maintenance of heterochromatin in fission yeast. *J. Biol. Chem.* **286**, 9308–9320