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Mechanisms of functional promiscuity by HP1 proteins

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Heterochromatin protein 1 (HP1) proteins were originally identified as critical components in heterochromatin-mediated gene silencing and are now recognized to play essential roles in several other processes including gene activation. Several eukaryotes possess more than one HP1 paralog. Despite high sequence conservation, the HP1 paralogs achieve diverse functions. Further, in many cases, the same HP1 paralog is implicated in multiple functions. Recent biochemical studies have revealed interesting paralog-specific biophysical differences and unanticipated conformational versatility in HP1 proteins that may account for this functional promiscuity. Here we review these findings and describe a molecular framework that aims to link the conformational flexibility of HP1 proteins observed *in vitro* with their functional promiscuity observed *in vivo*.

The multiple functions of HP1 proteins

The assembly of DNA into higher-order chromatin is central to the spatial and temporal regulation of the eukaryotic genome [1,2]. Several studies spanning more than a century have revealed two broad classes of chromatin domains: euchromatin, which contains gene-rich regions of the genome, and heterochromatin, which contains gene-poor and transcriptionally repressed regions [3–6]. At the core of the most conserved form of heterochromatin lies the HP1 protein [7]. The first HP1, HP1a, was identified in *Drosophila melanogaster* and was shown to localize to heterochromatin and act as a dominant suppressor of position-effect variegation (PEV) [8,9], a phenomenon in which there is non-uniform silencing of a euchromatic gene that is translocated into a heterochromatic region [10–13]. PEV was shown to require the spread of silencing activity by HP1a together with di- and trimethylation of lysine 9 on histone H3 (H3K9me2/3) [12–18].

Parallel to these genetic experiments, biochemical and structural characterization of HP1 proteins have helped provide molecular explanations for their roles in heterochromatin. These studies have identified multiple domains within HP1 proteins: the chromodomain (CD), which specifically recognizes the H3K9me2/3 mark; the chromoshadow domain (CSD), which forms a dimerization interface that recruits specific ligands; and a connecting hinge region, which interacts with nucleic acids [16,17,19–22] (Figure 1). Mammalian HP1 proteins have further been shown to interact with H3K9 methyltransferase and to oligomerize beyond dimers [23,24]. Collectively these studies have provided important starting points to explain how HP1 might participate in heterochromatin spread and chromatin condensation.

Although HP1 proteins were originally identified in the context of heterochromatin as reflected by their name, it is now clear that this family of proteins has additional nuclear functions including transcriptional activation and elongation, sister chromatid cohesion, chromosome segregation, telomere maintenance, DNA repair, and RNA splicing [25–39]. Consistent with the role of HP1 proteins outside of heterochromatin, the H3K9me2/3 mark, which helps recruit HP1 proteins, is also found in certain euchromatic regions [25]. The versatility of HP1 proteins can be explained in part by the fact that many eukaryotes have more than one HP1 paralog (Figure 1). Here, using language described in previous work, we use the term HP1 to define proteins containing a CD, a CSD, and a hinge region and having homology to the originally identified *Drosophila* HP1a (dHP1a) protein [40,41]. For example, humans possess three main HP1 paralogs – alpha (α), beta (β), and gamma (γ) – encoded by the CBX5, CBX1, and CBX3 genes, respectively. *Drosophila* possesses at least five paralogs (a, b, c, d, and e), whereas the fission yeast *Schizosaccharomyces pombe* has two paralogs (Swi6 and Chp2). As with their functions, the cytological distribution of HP1 paralogs is often distinct. For example, human HP1α (hHP1α) and hHP1β primarily associate with heterochromatic regions of the genome, such as centromeres and telomeres, and help mediate transcriptional gene silencing. By contrast, hHP1γ largely localizes to euchromatic regions and plays roles in transcriptional elongation and RNA processing [25,30,42,43]. Similarly, dHP1a is mainly associated with heterochromatin, whereas *Drosophila* HP1c helps regulate the transcription of genes in euchromatin [44].

Intriguingly, although HP1 paralogs perform different functions, they share high sequence homology. For example, in humans, the CD and CSD of HP1α and hHP1γ show 71% and 87% sequence identity, respectively (Figure 1). Nonetheless, it appears that these small sequence differences are

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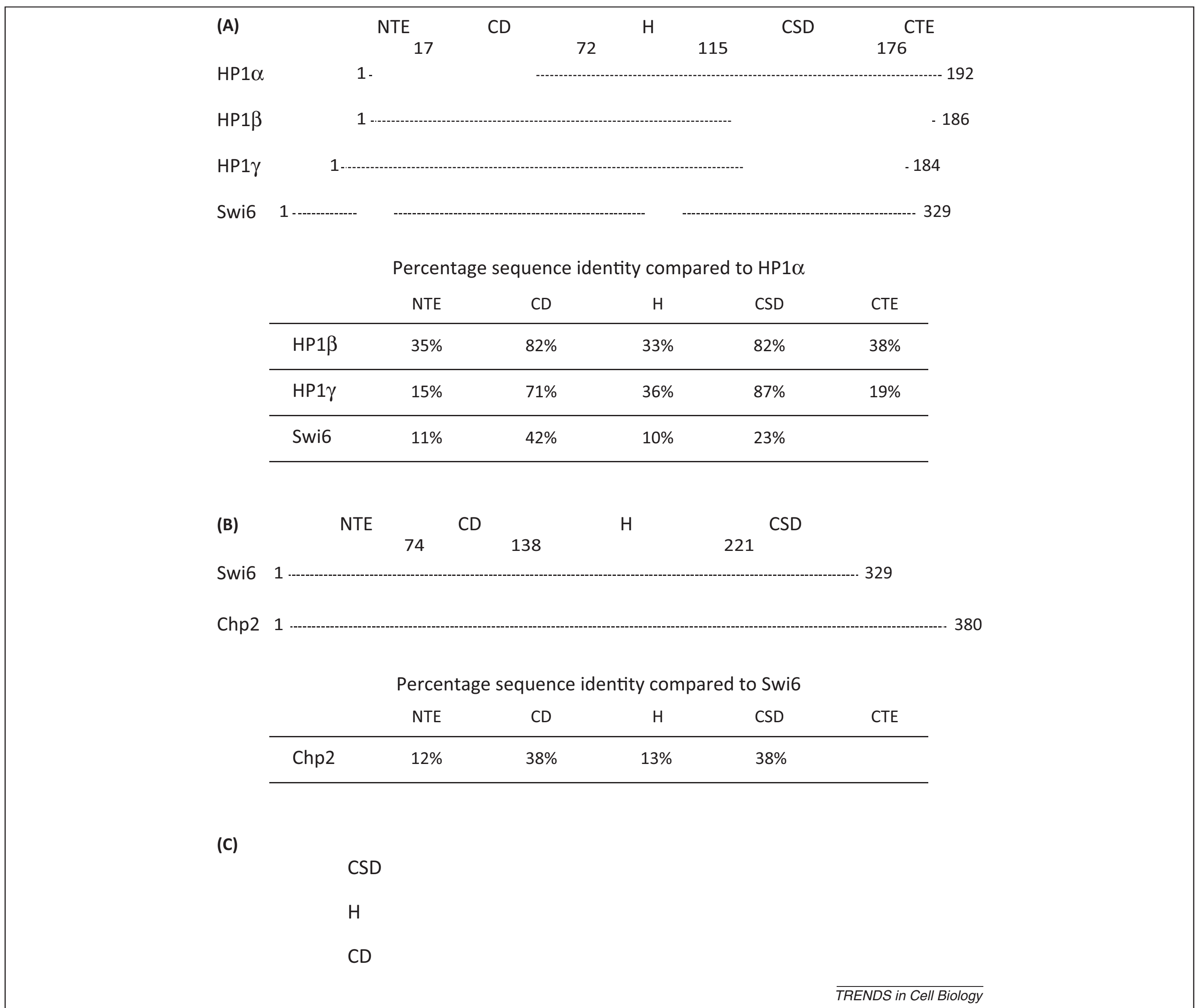

Percentage sequence identity compared to HP1α

	NTE	CD	H	CSD	CTE
HP1β	35%	82%	33%	82%	38%
HP1γ	15%	71%	36%	87%	19%
Swi6	11%	42%	10%	23%	

Percentage sequence identity compared to Swi6

	NTE	CD	H	CSD	CTE
Chp2	12%	38%	13%	38%	

Figure 1. Domain map and sequence identity of human and fission yeast heterochromatin protein 1 (HP1) proteins. **(A)** Top: Domain map of human HP1α, HP1β, HP1γ, and fission yeast Swi6. Bottom: Percentage sequence identity relative to human HP1α. **(B)** Top: Domain map of fission yeast Swi6 and Chp2. Bottom: Percentage sequence identity relative to Swi6. **(C)** Cartoon depicting the domain architecture of HP1 proteins. In (A), (B) and (C), light green indicates the N-terminal extension (NTE), yellow the chromodomain (CD), brown the hinge (H) region, blue the chromoshadow domain (CSD), and light red the C-terminal extension (CTE). In (C), only the CD, H, and CSD are shown for clarity. Sequence identity in (A) and (B) was calculated using the Needleman–Wunsch alignment method.

important, because domain-swapping experiments between different HP1 paralogs suggest that corresponding domains have specific and non-redundant functions [45–47]. Further, a single amino acid change in the CSD of dHP1a has been shown to change its specificity for ligand recognition [48,49]. These observations have led to the hypothesis that small deviations in sequence can result in large biochemical differences between HP1 paralogs that, in turn, translate into significantly distinct biological functions [27,41,43,50,51]. For these reasons, HP1 paralogs have previously been compared to histone variants where, despite 96% identity between H3.1 and H3.3 and 59% identity between H2A and H2AZ, the paralogs fulfill fundamentally different biological functions [41,52]. Just as with histone variants, the molecular basis for how small sequence changes in HP1 proteins cause large functional changes remains largely a mystery.

Although the above examples provide a rationale for how related HP1 paralogs can perform different functions, accumulating data also indicate that the same HP1 paralog participates in multiple functions. Consistent with this possibility, different populations of a given HP1 paralog, based on distinct on and off rates from chromatin, have been observed in mammalian and *S. pombe* cells [53–56]. A clear example is the multiplicity of roles attributed to the fission yeast HP1 protein Swi6 in gene silencing. Swi6 enables transcriptional silencing by recruiting silencing factors to reduce RNA polymerase occupancy [57–59] and post-transcriptional silencing by promoting the destruction of RNA transcripts [60,61]. Surprisingly, Swi6 can also interact with antisilencing proteins to limit heterochromatin spread [62,63]. Despite this accumulating evidence, how HP1 paralogs like Swi6 can fulfill multiple, and at times contrasting, roles is not well understood.

Our current understanding of the molecular basis for HP1 function is directly linked to the scope of the biophysical approaches that have been used to study HP1 behavior. In this review we use recent results derived from biophysical studies of *S. pombe*, mammalian, and *Drosophila* HP1 proteins, in the context of a wealth of previous observations, to suggest molecular explanations for the multiple functions of HP1 proteins. We describe how differences in the biophysical properties of HP1 paralogs can help explain their distinct biological roles. We further propose that conformational flexibility in HP1 proteins allows for their functional promiscuity *in vivo*.

Versatility of function from differences in sequence

The different HP1 domains (Figure 1) are thought to interact with specific regions of chromatin and with different ligands in a modular manner. Below we describe how sequence differences between these domains can alter the interactions made by the individual domains and thereby alter the functional properties of the corresponding HP1 proteins.

The CD and the N-terminal extension (NTE)

The CD contains a specialized hydrophobic cage formed by aromatic residues that bind methyl marks on histones with high specificity but low affinity. The CD of many HP1 proteins has been shown to specifically bind to H3K9me2/3 [16,17,64,65]; however, depending on the HP1 paralog, the affinity for an H3K9me3-tail peptide can span approximately 5–40 μM, as demonstrated for mouse HP1 paralogs (Figure 2A) [66]. This affinity difference provides a dynamic range that can be tuned in a paralog-specific manner for different functions. For example, phosphorylation of serine residues in the NTE of mouse HP1α increases its affinity for H3K9me3-tail peptides by approximately fivefold, bringing the overall affinity of the CD–H3K9me3 tail complex close to that of both mouse HP1β and mouse HP1γ [66]. Interestingly, in both mice and humans these serine residues are present only in the HP1α paralog (Figure 2B,C). In the HP1β and HP1γ paralogs, negatively charged glutamate residues replace some of the serines. Further, hHP1γ has fewer acidic residues in its NTE than hHP1α and hHP1β. Molecular dynamics studies suggest that these differences make the HP1γ CD–H3K9me3 complex more flexible than the HP1β CD–H3K9me3 complex [67]. The corresponding region of the NTE of the fission yeast homolog Swi6 does not contain the serines found in hHP1α, but instead contains several acidic residues that have been shown to stabilize binding to the H3K9me3 tail [68].

The above observations suggest that the presence of a negative charge in the NTE increases the affinity of HP1 proteins for methylated chromatin. Therefore, regulating the degree of negative charge via phosphorylation of the serines in hHP1α may allow the assembly of hHP1α on H3K9me2/3 chromatin to increase or decrease rapidly based on the activity of kinases or phosphatases, respectively. This property may be needed more for HP1α molecules, which are predominantly found in heterochromatic regions and may have to undergo coordinated assembly

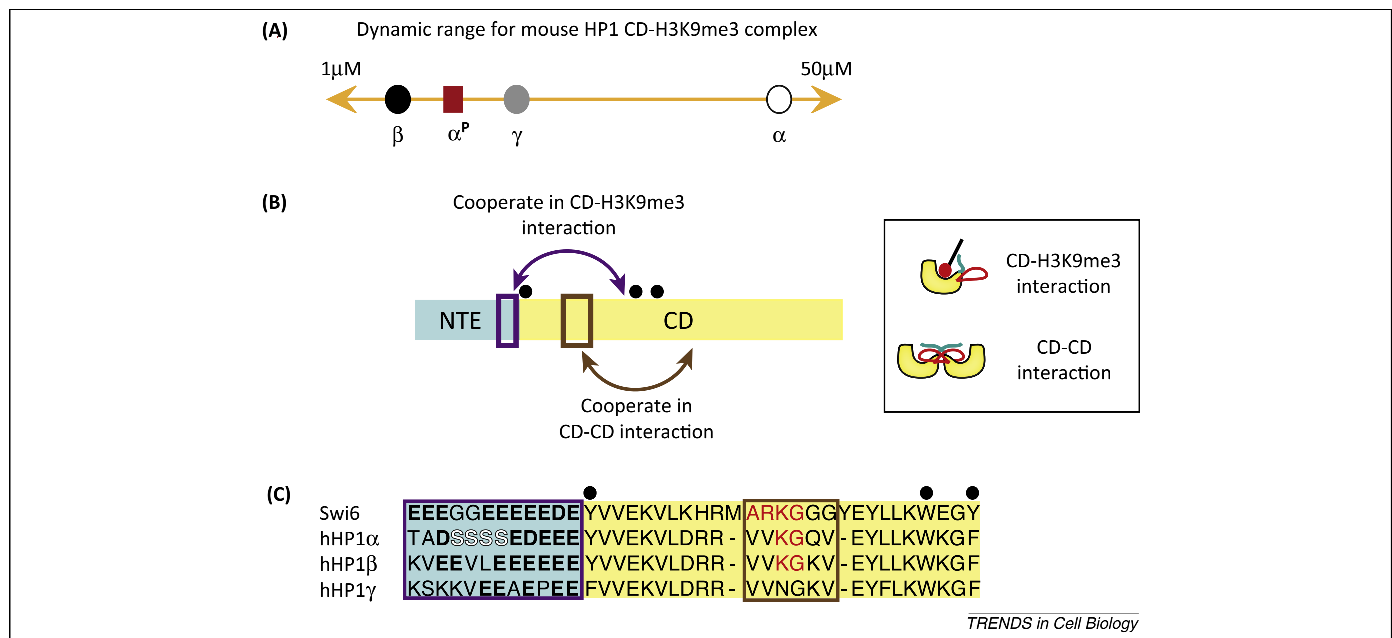

Figure 2. Mechanisms controlling the functions of the chromodomain (CD). **(A)** Dynamic range of K_d for binding of a trimethylated lysine 9 on histone H3 (H3K9me3)-tail peptide to CDs from mouse heterochromatin protein 1 (HP1)α (white circle, K_d = 35 μM), HP1β (black circle, K_d = 5.8 μM), HP1γ (grey circle, K_d = 12 μM), and phosphorylated HP1α (red square, K_d = 8.3 μM) as measured in [67]. **(B)** The N-terminal extension (NTE) is in light green and the CD in yellow. Left panel: Diagram indicates cooperation between the NTE region (purple box) and the CD in mediating CD–H3K9me3 tail interactions and cooperation between the histone mimic loop (brown box) and the aromatic cage (black dots) in mediating CD–CD interactions. Right panel: Cartoons indicating the two types of CD-mediated interaction observed in the context of Swi6. The ARK loop in the CD of Swi6 is in red. The H3 tail is in black, with the H3K9me3 mark shown as a red circle. **(C)** Sequence alignment between Swi6, human HP1α (hHP1α), hHP1β, and hHP1γ indicates the conservation and divergence of the key residues that mediate the CD–H3K9me3 tail and CD–CD interactions. Interactions between the aromatic cage and H3K9me3 mark are conserved across all HP1 isoforms. The interaction between the NTE and the H3 tail is shown for Swi6 and HP1α. Interaction between the ARK loop and the aromatic cage is shown for Swi6. Color coding is the same as in the left panel of (B). Negatively charged residues regulating the CD–H3K9me3 tail interactions in Swi6 and hHP1α are in bold. Phosphorylatable serines in hHP1α regulating its CD–H3K9me3 tail interaction are in white. The loop residues conserved with Swi6 are in red.

and disassembly during developmental transitions and the cell cycle. Interestingly, NTE phosphorylation of mammalian HP1α persists during the cell cycle [66]. We speculate, however, that small changes in the levels of phosphorylated HP1α may have large effects given the proposed cooperative nature of heterochromatin spread. The results with mammalian HP1α proteins are consistent with earlier *in vivo* studies in *Drosophila* showing that reversible phosphorylation of HP1a is essential for heterochromatin function [69,70].

For some HP1 proteins, the CD also helps mediate HP1 oligomerization, a key process in the spread of heterochromatin [24,68,71,72]. In particular, recent work has shown that the fission yeast Swi6 CD contains a sequence ($ARK_{94}GGG$) on a loop that resembles the amino acid sequence of the H3 tail surrounding the K9 position (ARK_9STG) (Figure 2B) [68]. Mutagenesis of the loop residues showed that the sequence promotes CD–CD interactions in solution, suggesting that the loop–CD interaction mimics the H3K9me3–CD interaction [68]. Interestingly, in the context of a Swi6 dimer, the loop–CD interaction blocks H3K9 methyl mark recognition and higher-order oligomerization. Binding to methylated nucleosomes via the CD pays the energetic cost for switching Swi6 dimers to a spreading competent state [68]. In this state, the vacant CDs can then either directly bind adjacent nucleosomes or bind the CD of another dimer via the $ARK_{94}GGG$ loop (Figure 4).

Although the lysine and proximal glycine in the histone mimic loop of Swi6 are conserved, the rest of the sequence degenerates in higher organisms (Figure 2C). Thus, it remains unclear whether the specific mechanism of CD–CD interaction observed in the fission yeast Swi6 is conserved in higher eukaryotes. However, the conserved lysine in hHP1α and β is differentially modified: it is monomethylated in hHP1α and acetylated in hHP1β [73]. Human HP1γ, by contrast, lacks the corresponding lysine. Post-translational modification of this conserved lysine has the potential to dramatically alter the basic biochemical properties of HP1α and β to promote a rapid switch between different biological functions. Although HP1 proteins preferentially recognize trimethylated H3K9, the affinity of the CD for monomethylated H3K9 tails has been shown to be least tenfold higher than unmethylated H3K9 tails in two different contexts [57,74]. We therefore speculate that, if the loop in hHP1α plays similar roles to that in Swi6, monomethylation of the loop lysine would increase its affinity for another CD and energetically compensate for the loss of the remaining histone mimic residues found in Swi6. Monomethylation of the lysine in hHP1α, in principle, could promote CD–CD interactions between two hHP1α proteins enabling oligomerization or provide a non-histone binding target for other CD-containing proteins (Figure 4B). As with phosphorylation, we speculate that such modulation may be needed more for hHP1α than for other paralogs because the assembly and disassembly of hHP1α needs to be coordinately regulated over large stretches of the genome. The methylation status of the corresponding loop lysine in Swi6 is unknown. Lysine acetylation, by contrast, could allow hHP1β to interact with bromodomain-containing proteins or could disrupt potential CD–CD interactions between HP1 molecules. Because hHP1β has both gene-silencing and gene-activating roles, acetylation may provide a means to switch between these two opposing roles by changing the oligomerization properties and binding partners of hHP1β. Analogously, we speculate that the absence of lysine in the corresponding loop region of hHP1γ may reduce its oligomerization across long stretches of chromatin, thereby enabling hHP1γ to function as a gene activator by exerting a more local effect.

The CSD and the C-terminal extension (CTE)

The CSD is involved in homodimerization of HP1 proteins and is a hub for many partners of HP1 [19,20]. In *Drosophila*, swapping the CSDs of dHP1a and dHP1c directs dHP1c to heterochromatin and dHP1a to euchromatin [75]. These results suggest that functional specificity of HP1 homologs in the cell relies, at least in part, on the ability of different CSDs to mediate association with specific nuclear proteins.

In general the CSD dimer of HP1 proteins is capable of reading PxVxL pentapeptide motifs, as well as sequences that partially deviate from it such as the PGTVAL sequence on the αN1-helix of histone H3, the LSVKI sequence on the *Drosophila* HP2 protein, and the PRVKV sequence in the *Drosophila* PIWI protein [20,48,49,76–78]. Recognition of PXVXL-type sequences relies on a set of conserved residues in the CSD (Figure 3A). The valine (V) at the center of the PXVXL motif (position 0) binds a conserved hydrophobic pocket in the dimer interface formed by tyrosine 168 (Y168) and leucine 172 (L172) (amino acid numbering for hHP1α). The plasticity of the binding site around the proline at position –2 allows other large hydrophobic groups, such as leucine or methionine, to substitute for it [79] (Figure 3A). However, if recognition of a PxVxL-type sequence relies on conserved residues in the CSD, how does a given CSD discriminate between different PxVxL sequences and how do different CSDs achieve different sequence specificities? Recent biochemical and structural studies with peptide ligands in solution have provided some insights into this question.

The residues upstream and downstream of the PXVXL pentapeptide motif (Figure 3A, –5 to –7 and +5 and +6, respectively) have been shown to act as key specificity determinants [79,80]. This is best illustrated by recent studies with dHP1a, which prefers to bind the HP2 protein over the PIWI protein. This preference relies, in part, on a conserved leucine in the CSD, which recognizes amino acids at positions –7/–6 and +5/+6 with respect to the LSVKI sequence in the HP2 protein [48,49]. These residues are missing in the amino acids flanking the PRVKV sequence in the PIWI protein. As a result, the CSD of dHP1a binds the LSVKI-containing sequence in the HP2 protein 50-fold tighter than the PRVKV-containing sequence in the PIWI protein. Substitution of this leucine with a lysine reduces the affinity of the dHP1a CSD for the LSVKI-containing sequence in HP2 by approximately 20-fold, while reducing the affinity of the PRVKV-containing sequence in PIWI by approximately 1.6-fold [49]. These differential effects reduce the preference of the CSD for the LSVKI- versus PRVKV-containing sequence and demonstrate how a single amino acid can be responsible for dHP1a's partner specificity.

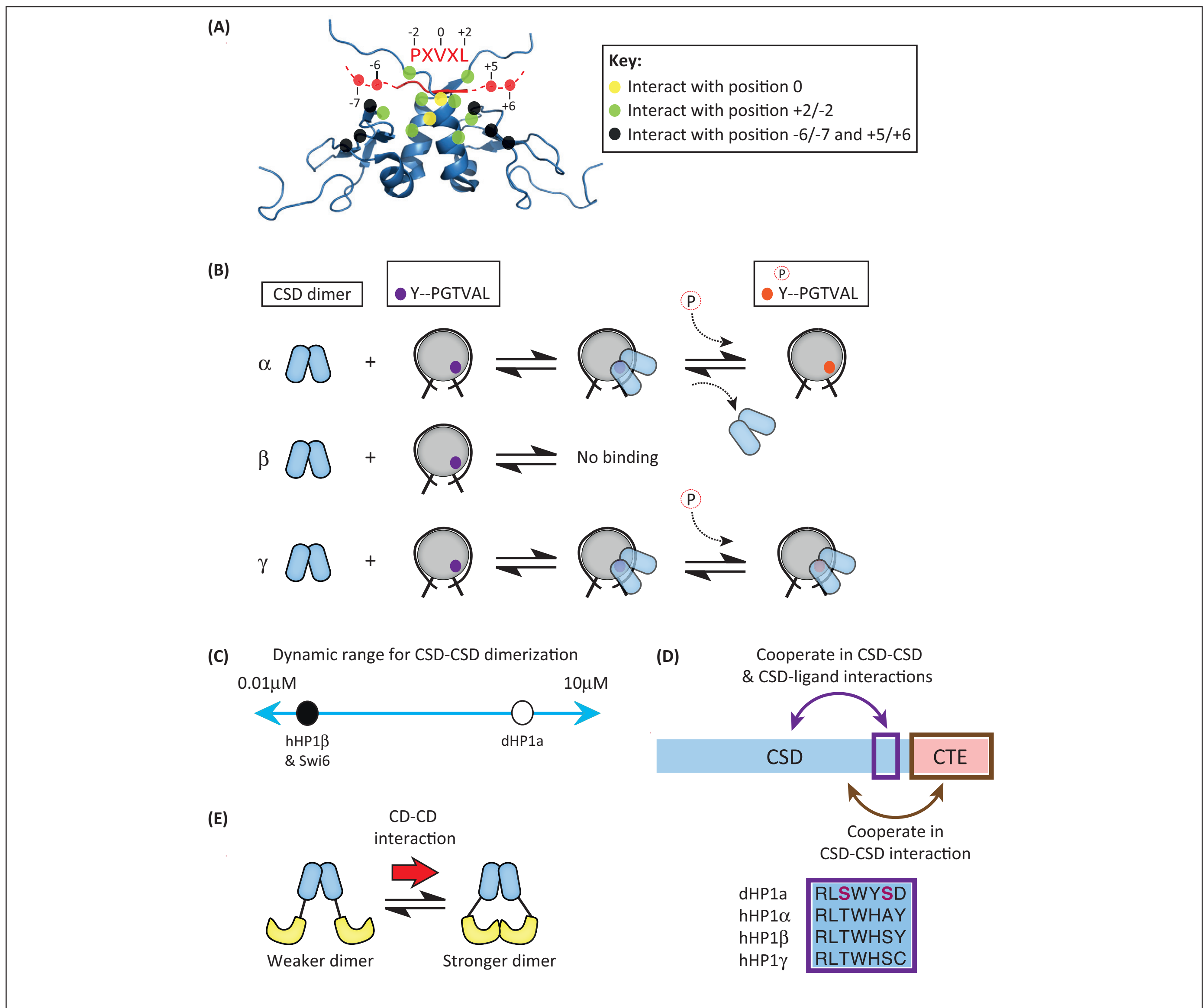

Figure 3. Mechanisms controlling the functions of the chromoshadow domain (CSD). **(A)** Structure of the CSD of mouse heterochromatin protein 1 (HP1)β (blue) in complex with the CAF (PXVXL) peptide (red), constructed using Protein Data Bank (PDB) file 1S4Z [80]. The numbering of the PXVXL-containing ligand is relative to the central valine (V) and the +5, +6, –6, and –7 residues are indicated with red circles. The residues in the CSD dimer that specifically interact with the different positions in the PXVXL peptide are shown as green and black circles. For clarity, the CSD residues that interact with position 0 of a PXVXL peptide are shown on only one of the two dimers as yellow circles. **(B)** Cartoon depicting how the CSD of human HP1α, β, and γ may differentially recognize the PGTVAL motif on histone H3 and the phosphorylation of H3Y41. For clarity, only one copy of the PGTVAL sequence on the nucleosome is shown. **(C)** Dynamic range of CSD–CSD dimerization constants measured for human HP1β (black circle), Swi6 (black circle), and *Drosophila* HP1a (dHP1a) (white circle). **(D)** Diagram indicating functional and structural cooperation between the C-terminal extension (CTE) region (brown box) and the CSD of HP1 proteins in mediating CSD–CSD and CSD–ligand interactions. The region of CSD containing the phosphorylatable serines in dHP1a (purple box) that regulate CSD–CSD dimerization and CSD–ligand interaction. The sequence of this region is compared with that in human HP1 proteins below. **(E)** Cartoon depicting how, in Swi6, the chromodomain (CD) can contribute to strengthening the dimerization of the full-length protein. The CD–CD interaction shown here is the one shown in Figure 2B (inset box) and is mediated by the ARK loop in Swi6.

Similarly, hHP1α and γ but not β bind to the PGTVAL motif on the αN1 helix of H3 (Figure 3B). HP1α additionally recognizes tyrosine 41 (Y41), which is two residues upstream from the PGTVAL motif. Phosphorylation of Y41 inhibits binding by HP1α to the PGTVAL motif in H3 [76,77]. By contrast, hHP1γ does not seem to require Y41 for binding the PGTVAL region and therefore it has been suggested that binding of HP1γ to H3 may be insensitive to Y41 phosphorylation [76,77] (Figure 3B). Although the molecular nature of such specificity remains elusive, the results make sense from a biological perspective because phosphorylation of this tyrosine in H3 is correlated with increased transcription. Furthermore, these results imply that, in the context of chromatin, non-histone ligands of the CSDs of hHP1α and γ would have to compete with the PGTVAL sequence in histone H3.

Because ligand recognition by the CSD requires CSD dimerization, the strength of dimerization can further tune the affinity for ligand. If the cellular concentration of a particular HP1 paralog is lower that its K_d for dimerization, a regulatory process that strengthens dimerization or increases local concentration can increase the affinity of the CSD interface for its ligands. The dimerization values of CSD–CSD from different HP1 paralogs range from low nanomolar for the CSD in Swi6 and hHP1β to micromolar for the CSD in dHP1a (Figure 3C) [48,49,68,72,79].

Analogous to how the NTE in the CD regulates CD–CD and CD–H3K9me3 tail interactions, the CTE in the CSDs of *Drosophila* HP1 paralogs has been shown to regulate interactions between two CSDs as well as interactions between the CSD–CSD dimer and its ligands (Figure 3D) [48,49]. The CTE length can be quite variable and is only four residues long in dHP1a, but 87 residues long in dHP1b. In dHP1b, the CTE significantly impairs CSD–CSD dimerization, lowering the dimerization affinity by approximately 100-fold, whereas the CTE in dHP1a contributes by increasing its affinity for specific CSD ligands [48,49]. The dimerization of Swi6 can also be controlled by the CD–CD interface as described above (Figure 3E), resulting in a tenfold increase in dimerization of the full-length Swi6 relative to the CSD alone [68]. The CD–CD interface can thus provide an additional location from which to regulate the dimerization of the CSD–CSD interface. This mechanism is in contrast to dHP1a, for which the dimerization of the CSD–CSD interface and the full-length protein are similar, suggesting that other domains do not participate in the dimerization of the full-length dHP1a protein [48].

Specific post-translational modifications in the CSD can also modulate ligand affinity. For example, phosphorylation of serines in the dHP1a CSD decreases dimerization by approximately sixfold but increases overall binding to PXVXL-containing ligands by approximately tenfold [48]. This argues that the affinity of the phosphorylated dimer is approximately 60-fold tighter for the PXVXL ligands.

The hinge region

Compared with the CD and CSD, the hinge region is more diverse and less conserved among HP1 proteins. The length of the hinge region also varies substantially between paralogs (Figure 1). The hinge region contains a nuclear localization sequence and is the site of several different post-translational modifications identified in HP1, from phosphorylation to SUMOylation [73,81–83]. Analogous to the CD and CSD, the hinge is implicated in interactions with different types of ligand including RNA and DNA, histone deacetylases, the inner centromere protein (INCENP), and components of the Origin Recognition Complex (ORC) [21,22,60,68,84–86] and these interactions are thought to help confer specific functions on the HP1 protein.

Because of its weak sequence conservation and its variable length, the hinge region represents an important source of functional differentiation between HP1 paralogs (Figure 1). Human HP1$\alpha$and γ have different affinities for RNAs *in vitro* and these could arise due to differences in the hinge regions of these proteins [21]. The molecular basis for this difference likely resides in the non-conserved residues because the critical charged residues responsible for nucleic acid binding (KRK and KKK) in the hinge region are conserved between all three mammalian HP1s. *In vivo*, site-specific post-translational modifications have been suggested to account for paralog-specific nucleic acid binding. For example, SUMOylation of residue 84 in the hinge region of mouse HP1α appears to promote the association of HP1α with RNA transcripts and specifically target HP1α but not HP1β or γ to pericentromeric regions of the genome [81]. In the case of human HP1γ, phosphorylation of serine 83 in its hinge region increases its interaction with Ku70, a central DNA repair protein, and increases its specific localization to euchromatin [87].

Finally, results with Swi6 raise the possibility that binding via the hinge region to DNA and RNA may have dramatically different biochemical effects. DNA enhances binding of Swi6 to the H3K9me3 mark whereas RNA antagonizes binding to the H3K9me3 mark [60,68], suggesting that differential recognition of DNA and RNA by the hinge region may allow Swi6 to switch between its roles in transcriptional and post-transcriptional silencing. This switch may occur because transcriptional silencing requires Swi6 to be associated with H3K9-methylated chromatin whereas post-transcriptional silencing entails dissociation of Swi6–RNA complexes from chromatin [60].

Together, the data presented in this section show how small changes in sequence between HP1 paralogs may enable different biological functions by affecting ligand recognition of the individual domains.

Versatility of function from conformational flexibility

Numerous enzymes and scaffold proteins involved in orchestrating signaling events display functional promiscuity [88–92]. It has been hypothesized that proteins that pilot more than one signaling pathway achieve their different functions by exhibiting some degree of conformational flexibility [88–92]. Such flexibility allows one molecule to generate diverse functional states that can be regulated by specific protein partners. The binding of these protein partners ultimately promotes a conformational change in the signaling molecule resulting in the switching on or off of a particular cascade of events in a network. The magnitude of conformational diversity of signaling molecules can range from small fluctuations of side chains to more global tertiary structure rearrangements. In either case the conformational flexibility provides a mechanism by which a single protein can achieve functional versatility. For example, the adenovirus early region 1A (E1A) oncoprotein is involved in cell cycle regulation and epigenetic cell reprogramming and interacts with diverse protein partners by means of its intrinsic structural adaptability. However, binding of any one molecular partner of EA1 results in its allosteric activation toward only one specific signaling pathway [93]. We propose that conceptually similar mechanisms might generate functional promiscuity within a single HP1 protein.

Conformational flexibility within an HP1 protein

NMR-based structural studies of human HP1β reveal not only that the hinge region is highly flexible and unfolded, but that the CD and CSD show significant conformational dynamics in solution [94]. In particular, the CD appears to sample more than one structural state in addition to the canonical H3K9me3-binding conformation [94]. However, the roles of the other conformations remain unknown. Nonetheless, these observations raise the possibility that ligands other than chromatin may stabilize alternative conformations and promote specific functional states.

In the case of Swi6, at least two distinct structural states have been identified: closed and open [68]. Mediated by the CD–CD interaction described above (Figure 2B), the closed

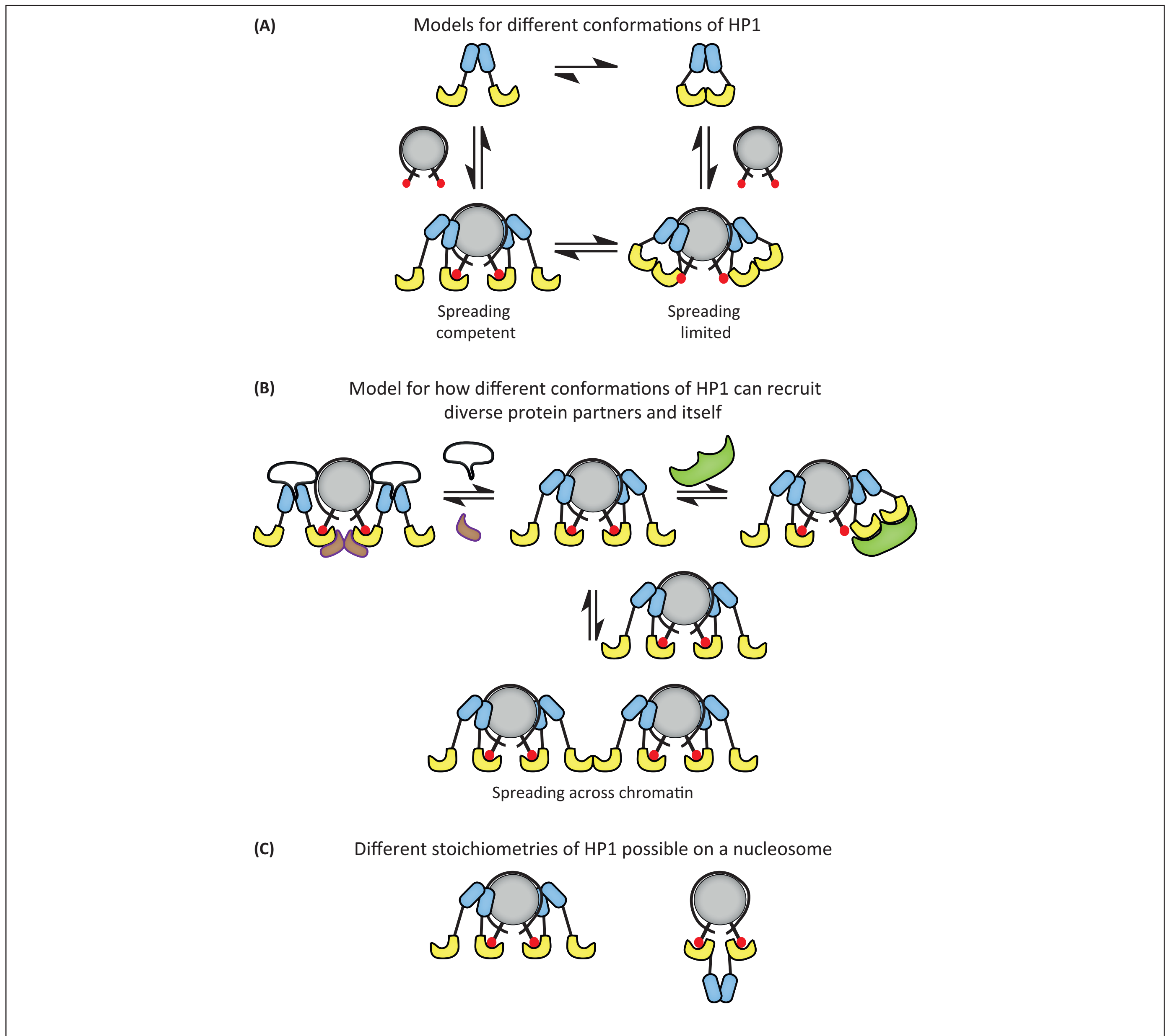

Figure 4. Models for functional versatility of heterochromatin protein 1 (HP1) on chromatin. **(A)** Cartoon depicting how different HP1 conformations can associate with nucleosomes. The spreading-competent and spreading-limiting conformations are based on recent findings with Swi6. **(B)** Cartoon depicting how different conformations of HP1 on nucleosomes can recruit different protein partners and itself. The white, brown, and green shapes refer to possible ligands of HP1 proteins bound to chromatin. The white shape is a chromoshadow domain (CSD) ligand, the brown shape is a putative interactor with the chromodomain (CD) loop, and the green shape is a putative capping protein that stabilizes HP1 in a closed state. **(C)** Cartoon depicting different possible stoichiometries of HP1 molecules on a nucleosome. The nucleosome depicted here contains a histone octamer (grey), DNA (black), and a trimethylated lysine 9 on histone H3 (H3K9me3) mark (red). Only the CD (yellow), the hinge region (black line), and the CSD (blue) of HP1 are shown for clarity. In (A) and (B), the depicted CD–CD interaction is mediated by the CD–ARK loop recently found to occur in Swi6 and described in Figure 2B. For clarity the ARK loop is not shown here.

state inhibits binding of the H3K9 methyl mark whereas the open state is capable of H3K9 methyl mark binding and spreading across chromatin via oligomerization (Figures 4A,B). We speculate that different conformations of Swi6 allow for different types of interactions with chromatin and other ligands. For example, although the spreading-competent state may promote Swi6 assembly on chromatin, the closed, autoinhibited conformation may serve to disengage Swi6 from chromatin. Binding by RNA appears to disengage Swi6 from methylated chromatin *in vivo* [60] and we speculate that RNA binding drives Swi6 into the closed state. The closed state may also be stabilized by other chromatin-binding proteins that help cap heterochromatin spread at boundary regions. For example, it has been shown that the antisilencing protein Epe1 needs to be recruited by Swi6 to limit heterochromatin spread [62,63]. Given that the closed state does not recognize the H3K9me mark, it is also possible that this conformation binds euchromatin through interactions with the nucleosomal DNA and possibly the PGTVAL sequence in histone H3 (Figure 4).

Conformational flexibility of HP1 domains on a nucleosome

The modular nature in which individual HP1 domains bind a nucleosome raises the possibility that different types of HP1–nucleosome complex are formed depending on which

HP1 domains are engaged with the nucleosome and which HP1 domains are engaged with other ligands (Figure 4B). In the case of Swi6, four molecules bind one H3K9-methylated nucleosome [72]. This architecture allows the formation of two unoccupied CDs that can bind nearby nucleosomes (Figure 4A,B). The interaction of Swi6 with a nucleosome is stabilized by multiple contacts made through the CD, the hinge region, and the CSD. Together these interactions increase the stability of a Swi6–H3K9me3 nucleosome complex approximately 100-fold over a Swi6–H3K9me3-tail peptide complex ($K_d = 10\ \mu M$ versus 100 nM, respectively, for peptide versus nucleosome) [68]. Studies of hHP1α on tetranucleosomes analogously suggest that the hinge and the CSD both help recognize methylated nucleosomes [95]. We hypothesize that the presence of multiple contacts between HP1 molecules and a nucleosome provides sufficient binding energy to allow individual domains to interact with other ligands while remaining associated with chromatin. Thus, a CSD dimer could interact with a PxVxL-containing ligand while the CD and hinge region provide the contacts to remain bound to chromatin. Similarly, the histone mimic sequence in the CD of Swi6 could interact with other CD-containing proteins while Swi6 remains bound to chromatin. Such mechanisms would allow different types of functions to be recruited to the HP1–nucleosome complex and help explain how the same HP1 paralog can perform different biological roles.

In contrast to Swi6, NMR-based studies imply that only one dimer of human HP1β binds per nucleosome [96]. Further, hHP1β seems to bind methylated nucleosomes with an affinity similar to methylated H3-tail peptides [96], arguing that, unlike Swi6 and hHP1α, hHP1β contacts only the H3K9me3 tail and no other nucleosomal surface. In this context, one can imagine that the CSD–CSD dimer interface of hHP1β is perhaps more readily available for binding PxVxL-containing ligands than the CSD–CSD interface of hHP1α.

The above observations illustrate how the same HP1 molecule has the potential to adopt different functional conformations *in vivo*. In addition, the different stoichiometries adopted by different HP1 paralogs on chromatin impose different constraints on the ability of these molecules to oligomerize and recruit other factors. Finally, given that the CSD can dimerize and, in certain cases like Swi6, the CD can dimerize, it is possible that different HP1 paralogs form heterodimers. Indeed, coimmunoprecipitation-based studies have suggested that mammalian HP1 paralogs can form heterodimers in solution as well as on chromatin [97]. The formation of such heterodimers can further diversify the structures that can be adopted by HP1–chromatin complexes and the types of ligands that are recruited to these complexes. However, it remains to be determined what regulates the formation of various HP1–nucleosome complexes and what their functions are in the cell.

Concluding remarks

Although HP1 proteins were originally thought to be structural proteins important for the integrity of heterochromatin, a wealth of data has shown that these proteins are also active participants in diverse nuclear activities. The studies reviewed here suggest that the diversity in sequences and conformations of HP1 proteins can help explain their different biological functions. It is possible to imagine HP1 proteins as chromatin adaptor proteins that enhance the signaling of histone modifications by providing additional functionalities to the chromatin platform (Figure 4B). Similar to histone variants and histone modifications, these functionalities can be regulated by HP1 paralogs and post-translational modifications. However, compared with histone variants and histone modifications, we know substantially less about the molecular impact of HP1 paralogs and HP1 modifications. The biophysical studies conducted on HP1 proteins to date have been critical for bridging the gap between biological phenomena and molecular mechanism. Because the biological roles played by HP1 proteins are being shown to be increasingly complex, it is necessary to keep pushing our understanding of how HP1 paralogs engage with chromatin and non-chromatin ligands at a structural and thermodynamic level. This process can be accelerated by increasing the types of quantitative biochemical methods available to study HP1 interactions and by high-resolution structures of HP1–chromatin complexes. As we learn more about the biochemical properties of different HP1 paralogs, the types of thermodynamic concepts described here can clarify the functions of HP1 proteins *in vivo*.

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