Review

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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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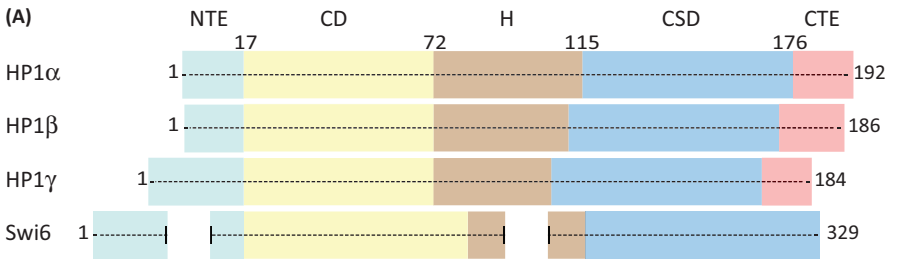
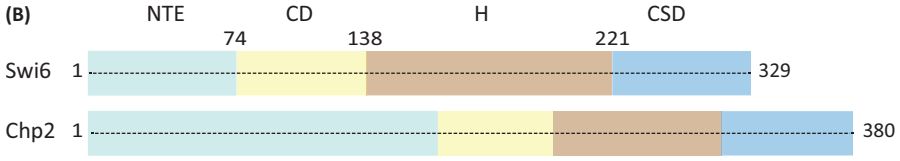


Percentage sequence identity compared to HP1α

	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%	

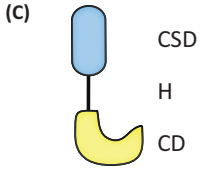


Figure 1. Domain map and sequence identity of human and fission yeast heterochromatin protein 1 (HP1) proteins. **(A)** Top: Domain map of human HP1α, HP1β, HP1γ, and fission yeast Swi6. Bottom: Percentage sequence identity relative to human HP1α. **(B)** Top: Domain map of fission yeast Swi6 and Chp2. 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.