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Linking Heterochromatin Protein 1 (HP1) to cancer progression

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Abstract

All cells of a given organism contain nearly identical genetic information, yet tissues display unique gene expression profiles. This specificity is in part due to transcriptional control by epigenetic mechanisms that involve post-translational modifications of histones. These modifications affect the folding of the chromatin fiber and serve as binding sites for non-histone chromosomal proteins. Here we discuss functions of the Heterochromatin Protein 1 (HP1) family of proteins that recognize H3K9me, an epigenetic mark generated by the histone methyltransferases SU(VAR)3-9 and orthologues. Loss of HP1 proteins causes chromosome segregation defects and lethality in some organisms; a reduction in levels of HP1 family members is associated with cancer progression in humans. These consequences are likely due to the role of HP1 in centromere stability, telomere capping and the regulation of euchromatic and heterochromatic gene expression.

Keywords

Cancer; Chromatin; Epigenetics; Gene regulation; Heterochromatin; Histone modifications

1. Introduction

Completion of the genomic projects for many organisms has provided a wealth of information on the organization of genes within genomes. The current challenge is to understand the mechanisms by which genes are activated and repressed in response to developmental programs and in disease states. A central component in transcriptional control is chromatin structure [1]. The fundamental packaging unit of chromatin is the nucleosome, comprised of ~146 bp of DNA wrapped around an octamer of histones [2]. Deciphering the rules that regulate chromatin packaging includes understanding the function of post-translational modifications that occur on histones. The fact that such modifications are reversible [3] offers the potential for therapy.

Eukaryotic genomes are packaged into two major types of chromatin: euchromatin and heterochromatin [4]. These types of chromatin are distinguished by sequence content, histone modifications and associated chromosomal proteins. Gene-rich euchromatin is packaged with nucleosomes containing acetylated histones and histone H3 methylated at lysine 4 (H3K4me), an epigenetic mark enriched at promoters of genes bearing RNA polymerase II. In contrast, gene-poor heterochromatin is packaged with nucleosomes containing hypoacetylated histones and histone H3 methylated at lysine 9 (H3K9me) and histone H4 methylated at lysine 20 (H4K20me) [3]. Modifications of histone tails alter chromatin packaging and/or protein–protein interactions regulating processes that occur on the chromatin template.

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Several proteins have been identified that bind specific modifications on histone tails [3]. One example is Heterochromatin Protein 1 (HP1), an evolutionarily conserved non-histone chromosomal protein enriched within heterochromatin and present at specific sites within euchromatin [5-9]. HP1 proteins form a family in which each member possesses two conserved domains: a chromo domain (CD) and a chromo shadow domain (CSD) separated by a flexible hinge (Fig. 1) [10]. The first member of this family to be identified was *Drosophila* HP1, sometimes referred to as HP1a [8,11,12]. Other family members in *Drosophila* include HP1b-e, each possessing the characteristic domain organization, yet exhibiting different functions, expression profiles and/or chromosomal localization patterns [12]. The mouse genome encodes three HP1 family members, HP1alpha, M31 (HP1beta) and M32 (HP1gamma) that possess functions similar to *Drosophila* HP1 [13,14]. Likewise, the human genome encodes three HP1-like proteins, HP1^{Hsa}, HP1^{Hsβ} and HP1^{Hsγ}, which show partially overlapping chromosomal localization patterns and shared functions [15-17]. Inter-species studies showed that human HP1^{Hsa} rescues lethality associated with mutations of the *Drosophila* gene encoding HP1, *Su (var)2-5*, implying conserved function [18]. In contrast, mouse M31 does not complement yeast *swi6* mutants, suggesting species-specific functions of the CSD [19].

Here we summarize the complexities of HP1 localization and function in diverse nuclear processes. We highlight functional analyses derived from model organisms and mammalian cell culture. These studies have implications for understanding the role of HP1 in relation to human disease such as cancer.

2. Mechanisms of HP1 localization

HP1 proteins localize to centric heterochromatin, telomeres and specific sites within euchromatin [8,20]. The major mechanism of localization within centric heterochromatin is through modified histone tails. Structural studies revealed an interaction between the hydrophobic pocket of the HP1 CD and methylated lysine nine of histone H3 (H3K9me) (Fig. 1) [21]. This epigenetic mark is generated by a conserved family of histone methyltransferases named after the *Drosophila* member SU(VAR)3-9 [22,23]. Both HP1 and SU(VAR)3-9 play a role in heterochromatin initiation near centromeres that involves RNAi machinery [24]. Loss of SU(VAR)3-9 and related orthologues in *S. pombe*, *Drosophila* and mammalian cells, results in displacement of HP1 from centric regions and the loss of gene silencing within centric regions [25].

Mechanisms by which HP1 localizes to sites within euchromatin appear to involve more than recognition of H3K9me. Localization of HP1 by immunostaining of chromosomes and DamID experiments showed only partial overlap of HP1 and H3K9me, suggesting multiple mechanisms [26,27]. An alternative mechanism of localization might be through interactions between the CSD and other factors. Structural studies revealed that the HP1 CSD homodimerizes through an alpha helical region (Fig. 1) [28]. Dimerization generates a platform that interacts with proteins possessing the peptide sequence PxVxL [28], potentially eliminating the need for methylated histones in the localization process. This is an attractive model for localization of HP1^{Hsa} at genes bound by zinc finger KRAB domain proteins. These proteins silence genes by recruiting the transcriptional co-repressor TIF1β (or KAP1), which interacts with HP1 [29]. A second alternative mechanism of localization involves interactions with RNA, as association of HP1 with specific loci in *Drosophila* and centric regions in mouse are susceptible to RNase treatment [30,31]. A third mechanism for localization is observed at the distal ends of *Drosophila* telomeres, where HP1 is thought to directly bind DNA [32]. In humans and mice, however, HP1 proteins localize to telomeric regions via H3K9 association [33-36]. Therefore, HP1 appears to use information at the genetic level in the form of DNA sequence content and at the epigenetic level in the form of modified histones for localization.

3. HP1 contributes to centromere stability

Centromeres are specialized regions of eukaryotic chromosomes that direct sister chromatid segregation during mitosis [37]. Centromeres are packaged with a variant of histone H3 and other histones that possess specific post-translational modifications [38,39]. The outer surface of the centromere serves as a platform for the assembly of proteins that make up the kinetochore, which is the site of attachment for microtubules. Surprisingly, HP1 proteins and histone H3K9me are not found within the centromere proper, but in the flanking regions [38-40]. This peripheral localization appears to contribute to centromere function as loss of the *S. pombe* HP1 orthologue Swi6 causes chromosome segregation defects, with an increased occurrence of lagging chromosomes and chromosome loss [41]. Swi6 interacts with Psc3, a cohesin subunit, suggesting that the chromosome segregation defect observed in *swi6* mutants may be a result of the loss of cohesin subunits from centric regions [42,43]. These findings suggest that Swi6 recruits cohesin to centromeres [43].

In *Drosophila* embryonic cell culture, a reduction in the levels of HP1 causes chromosome segregation defects. These cells possess disorganized spindles at metaphase, chromosomes are mis-aligned during prometaphase, and chromatin bridges and lagging chromatids are apparent at telophase [44]. Collectively, these phenotypes are consistent with structural defects associated with the kinetochore. However, HP1 localizes to the promoters of cell cycle regulatory genes in these cells and expression of the mitotic kinase Aurora B is altered, suggesting that transcriptional as well as structural defects contribute to the phenotypes observed.

In mammalian cells, a reduction in the levels of HP1 produces chromosome segregation defects. Knock-down of both HP1^{Hsa} and HP1^{Hsy} by RNAi in HeLa cells abolishes localization of the HP1-interacting kinetochore protein hMis12 which results in aberrant chromosome segregation [45]. Mice lacking Suv39h1 and Suv39h2 show a reduction of HP1 at centric regions and have widespread genomic instability and increased incidence of lymphomas [46]. In addition, male mice show spermatogenic failure, most likely due to non-homologous chromosome associations. Collectively, these studies demonstrate that HP1 is required for centromere stability in a variety of organisms, perhaps to establish a chromatin structure that helps to distinguish centric regions for the specialized functions dedicated to centromeres.

The functional relevance of specific epigenetic modifications at centromeres has recently been investigated. In an elegant study, various proteins were targeted to the centromere of an artificial mini-chromosome; chromosome stability was used as a measure of kinetochore function [47]. Targeting a transcriptional activator caused chromosome segregation defects, whereas control proteins had no effect. Likewise, targeting the transcriptional co-repressor TIF1 β that recruits HP1 resulted in altered histone modifications and disruption of kinetochore function. Collectively, these data strongly suggest that both “open” and “closed” chromatin are incompatible with kinetochore function, which may require a specialized chromatin state. This idea is supported by cytological studies showing unique combinations of epigenetic marks at centromeres [39].

4. HP1 provides telomere stability

Most eukaryotic telomeres are defined by G-rich repetitive sequences. These ends are elongated by telomerase and protected from degradation by non-nucleosomal protein complexes [48]. A specialized chromatin structure at telomeres allows the cell to distinguish a natural chromosome end from a DNA double strand break. In *Drosophila*, an organism that lacks telomerase, telomeres are defined by arrays of retrotransposons. These chromosome ends are elongated by the addition of retrotransposons and protected by non-nucleosomal proteins that assemble at telomeres [49]. *Su(var)2-5* mutants show increased levels of expression from

the retrotransposons and telomeric fusions [50,51]. While HP1 appears to localize to the distal ends of chromosomes through interactions with DNA [32], localization within the retrotransposon arrays and within sub-telomeric associated sequences (TAS) appears to be through an interaction with H3K9me [52]. This mechanism of localization at telomeres is conserved. Mouse embryonic fibroblast cells lacking Suv39h1 and Suv39h2 exhibit reduced levels of H3K9me and HP1. These alterations in chromatin correlate with telomere elongation [33]. Loss of telomerase leads to reduced levels of HP1 and H3K9me. In addition, increased levels of telomeric H3 and H4 acetylation and gradual telomere shortening are observed [53]. Thus, HP1 plays a role in end capping, length stability and transcriptional control at telomeres.

5. HP1 and gene regulation

Null alleles of *Su(var)2-5* are lethal in *Drosophila*; heterozygotes are suppressors of position effect variegation (PEV) [54]. PEV is the silencing of euchromatic genes brought into juxtaposition with heterochromatin through transposition or chromosomal rearrangement [55]. Similarly, increasing the dosage of HP1 in mice caused silencing of a variegating reporter gene inserted near centric heterochromatin [56]. These findings strongly suggest that the levels of HP1 regulate the amount of heterochromatin, with HP1 playing a negative role in gene regulation. Consistent with these findings, targeting HP1 upstream of reporter genes integrated at euchromatic sites within the *Drosophila* genome caused the formation of heterochromatin domains and silencing of the reporter genes [57]. Targeting HP1 proteins upstream of reporter genes on transiently transfected plasmids in mammalian cell culture also resulted in gene silencing [58,59]. Thus, HP1 proteins are capable of nucleating heterochromatin and causing gene silencing at both centric regions and ectopic sites within euchromatin.

Chromosome four of *Drosophila melanogaster* provides an opportunity to study the role of HP1 in gene expression in an unusual chromatin environment. This small (~4.5 Mb) chromosome has ~88 genes distributed within a 1.2Mb region of alternating euchromatic and heterochromatic domains [60]. High-resolution chromatin immunoprecipitation (ChIP) analyses showed co-localization of HP1 with Painting of Fourth (POF) within exon genes on the fourth chromosome [61]. POF possesses motifs involved in RNA binding and is enriched on chromosome four relative to other chromosomes. Loss of HP1 resulted in increased expression of these genes, suggesting a negative role in transcription. In contrast, loss of POF resulted in decreased expression of these genes, implying a positive role for this factor in gene expression. Taken together, these data suggest antagonistic functions for POF and HP1 in the transcription of fourth chromosome genes.

In contrast to a negative role in gene expression, there is increasing number of examples in which HP1 proteins play a positive role in transcription. First, *Drosophila* HP1 is required for proper expression of genes that naturally reside within heterochromatin [62]. Second, HP1 is required for the expression of some euchromatic genes that directly associate with HP1 and possess H3K9me [63]. Surprisingly, these genes exhibit decreased expression in a *Su(var)2-5* mutant background, supporting a positive role for HP1 in expression. A third example of HP1 as a positive regulator of transcription comes from studies of activated genes in *Drosophila* [31]. HP1 localizes to heat shock and developmental “puffs” in polytene chromosomes that represent sites of intense gene activity. In the absence of HP1, these genes are no longer induced. Collectively these studies broadened the transcriptional role for HP1 to include activation and demonstrated that the presence of H3K9 methylation in conjunction with HP1 association cannot be used as an indicator of gene repression.

The role of HP1 in gene expression is complex and likely involves multiple mechanisms. As a silencing factor, HP1 associates with the promoter regions of genes and prevents transcription initiation by establishing a compact chromatin structure and/or recruiting histone deacetylases

and DNA methyltransferases [64,65]. As a factor that “dampens” gene expression, HP1 associates with the coding region of genes [61]. The mechanism of transcriptional dampening is unknown, but might involve regulation of elongation and/or the prevention of cryptic initiation behind the passing polymerase. As an activating factor, HP1 might serve to establish specific chromatin architecture. Heterochromatic genes are spread over large genomic regions, possess multiple exons and have repetitive elements within introns. Dimerization of HP1 bound at distant genomic sites could bring distal regulatory elements into close proximity of promoters. Another mechanism by which HP1 might serve a positive function is through RNA processing. The fact that HP1 association at developmental and heat shock loci in *Drosophila* is sensitive to RNase treatment suggests roles in RNA processing. The ability of HP1 to have different effects on gene expression might reflect changes in association with partner proteins. Additional biochemical analysis of HP1-containing complexes should provide insights on this issue.

6. HP1 and cancer progression

The development and progression of cancer is accompanied by changes in gene expression. These changes are often on a global scale and can be caused by both genetic and epigenetic alterations. Most transcription factors are not gene-specific; a defect in one factor can have consequences on the expression of many target genes. Defects in a chromatin packaging protein or an enzyme that modifies histones can alter the chromatin status of multiple genomic regions, simultaneously affecting the expression of hundreds of genes. Given the widespread genomic distribution of HP1 and its role in transcription, it is not surprising that HP1 has links with cancer.

Currently, no human diseases are associated with mutations in the genes encoding HP1^{Hsα}, HP1^{Hsβ} or HP1^{Hsγ}. However, changes in the levels of expression of these genes have been reported for many cancers (Table 1). Gene expression profiling studies revealed reduced levels of HP1^{Hsα} mRNA in papillary thyroid carcinomas compared to normal thyroid tissue [66]. Likewise, HP1^{Hsα} mRNA is reduced in cases of embryonal brain tumors from individuals with a poor prognosis, compared to those with more favorable outcomes [67]. Reduced levels of HP1^{Hsα} mRNA was among the best predictors of treatment failure for embryonal brain cancer [67].

Consistent with decreased levels of HP1 correlating with poor prognosis, increased levels of HP1^{Hsα} may correlate with a reduction in metastasis in colon cancer [68]. Colon cancer cells that over-express gastrin-releasing peptide (GRP) and its receptor (GRPR) coordinately up-regulate six proteins and their corresponding mRNAs, one of which is HP1^{Hsα} [68]. GRP and GRPR act as morphogens, promoting a differentiated state while limiting proliferation and spread. Thus, increased levels of HP1^{Hsα} could minimize cancer cell dissemination throughout the body.

HP1 appears to play a role in ovarian cancer progression. Evidence of this comes from studies of a heat shock protein 90 (HSP90) inhibitor [69]. HSPs are molecular chaperones that function to protect client proteins, such as transcription factors, from misfolding, aggregation and degradation, particularly under stress. 17-allylamino-17-demethoxygeldanamycin (17 AAG) is an HSP90-specific ATPase inhibitor that is currently being used in phase II clinical trials to prevent cancer progression. Loss of ATPase activity inhibits the protective function of HSP90 and leads to degradation of HSP90 client proteins. Protein analyses of ovarian cancer cells treated with 17AAG showed increased HP1^{Hsγ}, relative to control treated cells. These findings are consistent with an anti-cancer role for HP1^{Hsγ} [69].

While several studies have shown correlations between alterations in HP1 protein levels and cancer progression, studies in breast cancer have demonstrated a causal role for HP1^{Hsα}

regulating breast cancer cell invasion. HP1^{Hsa} is down-regulated in highly invasive/metastatic cells relative to poorly invasive/non-metastatic cells [70]. Consistent with these findings, levels of HP1^{Hsa} were decreased as much as 95% in metastatic tissues from breast cancer patients compared to levels present in primary breast cancer tumors [70]. Knock-down of HP1^{Hsa} in poorly invasive/metastatic cells increased *in vitro* invasion by 50% relative to controls (without altering cellular growth rates), demonstrating a causal role for HP1^{Hsa} in the invasion process. Likewise, expression of exogenous HP1^{Hsa} in highly invasive/metastatic cells decreased invasion by 30% relative to controls, with no effect on growth. The partial reversal of the invasive phenotype upon expression of HP1^{Hsa} in invasive/metastatic breast cancer cells was dependent upon HP1^{Hsa} dimerization [71]. Expression of a mutant form of HP1^{Hsa} possessing a single amino acid substitution (I165E, Fig. 1) that disrupts dimerization did not alter invasion. In contrast, a mutant form of HP1 possessing a single amino acid substitution (W174A, Fig. 1) that disrupts protein partner interactions altered invasion to the same extent as wild type HP1. Taken together, these data are consistent with HP1^{Hsa} functioning as a metastasis suppressor. Metastasis suppressors make up a growing class of proteins that regulate metastasis, without altering tumor growth [72]. This is in contrast to tumor suppressors that inhibit tumor cell growth and may or may not regulate metastasis.

A mechanism by which HP1^{Hsa} might regulate invasion/metastasis is through alterations in gene expression. Supporting this idea, hundreds of genes change expression upon knock-down of HP1^{Hsa} in a poorly invasive/non-metastatic breast cancer cell line (Moss and Wallrath, unpublished). The non-clustered arrangement of affected genes within euchromatin suggests that HP1^{Hsa} does not regulate gene expression on a domain basis. Gene ontology analysis revealed that many products of these genes have known connections to cancer and metastasis.

In contrast to the beneficial function that HP1 proteins appear to play in inhibiting cancer progression, there are examples in which HP1 proteins might have deleterious effects in cancer cells. In general, HP1 levels are relatively low in immune cells compared to most other types of cells. In fact, none of the three HP1 proteins or H3K9me are detected in neutrophil granulocytes, and only rarely observed in eosinophil granulocytes [73]. In contrast, elevated levels of all three HP1 proteins and H3K9me were detected in the granulocytes of acute myeloid leukemia patients and chronic myeloid leukemia (CML) patients in an acute phase of the disease [73,74]. These findings are reminiscent of those in *Drosophila* and mice where increased dosage of HP1 causes increases in heterochromatin formation as evidenced by increased gene silencing.

An inverse correlation between the levels of HP1 and the serpin monocyte and neutrophil elastase inhibitor (MNEI) exist in differentiated human granulocytes [74]. MNEI is related to the chromatin-condensing serpin, MENT protein, in chickens. The levels of HP1 and MNEI inversely correlate in the peripheral blood cells of both healthy and CML patients and during retinoic acid-induced differentiation of a leukemic monocyte lymphoma cell line. HP1 levels decrease and MNEI levels increase upon differentiation. These data suggest that MNEI may replace HP1 in heterochromatin during the terminal differentiation of granulocytes. Over-expression of HP1 in leukemias is then anticipated to cause global changes in gene expression and/or chromatin condensation. The detection of HP1 in granulocytes might serve as an indicator of potential oncological blood disorders.

7. The interplay among HP1, DNA and histone methylation

In mammalian cells, cytosine residues of CpG dinucleotides are methylated by DNA methyltransferases (DNMTs) [75]. There is a well-documented interplay between DNA methylation and H3K9 methylation in many organisms [76]. This connection is apparent in studies of human colon cancer cells. Cells lacking DNMT1 exhibit reduced levels of DNA

methylation at CpG dinucleotides within pericentric satellite 2 and 3 sequences, while global levels of DNA methylation are relatively unchanged [77]. Loss of DNA methylation occurs in conjunction with loss of HP1^{Hsa} and H3K9me at satellite 2 sequences. These changes correlated with gross morphological defects in nucleolar structure and heterochromatin disorganization.

While alterations in the patterns of DNA methylation are frequently observed in cancer, abnormal histone methylation patterns are also found [78]. The GASC1 protein, encoded by *gene amplified in squamous cell carcinoma 1*, is over-expressed in multiple types of cancers, including esophageal and prostate cancers. In addition, the genomic region containing *GASC1*, 9p23-24, is amplified in many types of cancer [79,80]. GASC1, also known as JMJD2C, contains two jumonji domains responsible for demethylating H3K9di-me and H3K9tri-me [79]. In addition, this factor possesses double PHD zinc finger and tudor domains that recognize methylated histones and are thought to play a role in targeting this enzyme [81]. Two closely related jumonji domain-containing proteins, JMJD2A and JMJD2B, possess similar demethylase activities and are over-expressed in prostate cancer. Importantly, H3K9 demethylation by any of these three proteins results in decreased chromatin association of all three HP1 isoforms [79]. Surprisingly, global changes in H3K9me were not detected in esophageal cells over-expressing GASC1, suggesting that this demethylase might be targeted to specific loci.

8. Utilization of HP1 by viruses

HP1 regulates specific steps within viral life cycles. For HIV-1, transcription of the genome regulates the rate of viral replication and AIDs progression. The viral genome contains nucleosome positioning sequences within the long-terminal repeat (LTR) that place nucleosomes over key regulatory elements [82]. Transcription initiation within the LTR requires disruption of the nucleosome and activation by the viral transactivator Tat [83]. During HIV-1 quiescence or latency, the integrated provirus in the host genome remains transcriptionally silent despite the presence of a paused polymerase at the promoter [84]. In T-cells, transcriptional silencing is due to histone methylation by Suv39H1 and association of HP1 proteins [85]. Knock-down of HP1^{Hsa} has no effect on transcription from the LTR, knock-down of HP1^{Hsb} activates transcription and knock-down of HP1^{Hsy} causes a slight decrease in expression [9]. These effects are thought to be direct, as HP1^{Hsb} is localized to the silenced HIV-1 LTR by ChIP analysis. Upon transcriptional induction, HP1^{Hsb} dissociates and HP1^{Hsy} binds, demonstrating a switching mechanism of transcription control.

In microglial cells, which are targets of HIV-1 infections within the central nervous system, latency is regulated by association of the cellular zinc finger protein COUP-TF-interacting protein 2 (CTIP2). CTIP2 is recruited by the transcriptional regulator Sp1 bound at the LTR [86]. CTIP2 possesses binding sites for both Tat and HP1^{Hsa}; formation of this three-factor complex correlates with localization of integrated HIV-1 to transcriptionally inactive regions near the nuclear periphery.

A role for HP1 in controlling viral latency has also been observed for human cytomegalovirus and Kaposi's Sarcoma-associated Herpesvirus (KSHV) [87]. In the case of Kaposi's Sarcoma-associated virus, HP1^{Hsa} represses transcription through an association with latency-associated nuclear antigen (LANA), a factor that binds to sequences within the terminal repeats. This interaction appears to be responsible for the subnuclear distribution of LANA (and perhaps the viral genome) to the nuclear periphery [88].

The role of HP1 in nuclear processes has expanded to include nuclear envelope (NE) functions. HP1 has a close relationship with the NE, most likely through interactions with the lamin B receptor (LBR) [89,90]. The NE forms a barrier for viral egress from the nucleus. To overcome

this barrier, the human JC virus (JCV), a member of the polyomavirus family responsible for progressive multifocal leukoencephalopathy, interferes with the interaction between LBR and HP1. The N-terminal region of the virally encoded protein agnoprotein (Agno) competes with LBR for binding to HP1 [91]. As a consequence of Agno binding to LBR, HP1 is displaced from the NE, resulting in increased mobility of LBR within the NE. The net effect is disruption of the NE, allowing for viral particles to be expelled from the nucleus and spread to neighboring cells. This effect is likely not specific for JCV, as agnoproteins of other viruses show amino acid sequence conservation with the N-terminus of JCV Agno.

9. Conclusion and Perspectives

The nuclear processes in which HP1 proteins are known to participate have increased in number and become more diverse, with many having connections to human disease (Fig. 2). Localization of HP1 proteins at the centric and telomeric regions impact chromosome stability in a variety of organisms. This is potentially relevant in human cells, as the levels of HP1 proteins are altered in many types of cancers where mis-segregation of chromosomes produces aneuploidy observed with cancer progression. In addition to roles in chromosome stability, new links are being made between HP1 and DNA replication, DNA repair and cell cycle checkpoints [92,93].

The traditional view that HP1 proteins silence transcription via heterochromatin formation needs to be expanded. Emerging data suggest multiple mechanisms for transcriptional repression. In some cases HP1 appears to function at promoters, whereas in other cases HP1 is required within the coding region of active genes. The mechanisms used for repression are not well understood but likely involve histone modifications and partner proteins. In addition to the classic gene repression function, HP1 proteins are required for proper expression of specific genes in euchromatin and heterochromatin. In these cases, the mechanisms by which HP1 promotes gene expression are completely unknown, possibly involving interactions with transcriptional elongation and RNA processing machinery. Given the broad distribution of HP1 proteins in the genome and these diverse roles in gene expression, altered levels of HP1 proteins in cancer cells are likely to have profound effects on global gene expression. Adding to the complexity, many anti-cancer therapy drugs currently in use alter epigenetic modifications that influence HP1 localization and function [94]. Therefore, an in-depth understanding of HP1 functions will provide additional targets for therapy and allow for better predictions of therapeutic outcome.

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Fig. 1.

HP1 domain organization and structure. (A) Schematic representation of mouse HP1alpha is shown with the chromo (CD) and chromo shadow (CSD) domains separated by the flexible hinge region. Amino acid substitutions known to affect protein function are indicated (V22M, H3K9me binding; I165E, dimerization; W174A, PxVxL interactions). (B) Ribbon diagrams derived from the crystal structures of the CD (blue) in complex with H3K9me2 (green; 1kna.pbd) and a dimer of the CSD (red) interacting with a PxVxL-containing peptide present in known interacting partners (green, 1s4z.pbd). Due to the flexible nature of the hinge region, no crystal structure is currently available.

Fig. 2. Interaction diagram for HP1 in nuclear processes. Colored spheres represent HP1 and nuclear processes that involve HP1. Overlap among the spheres indicates shared participation in a function; NE, nuclear envelope.

Table 1

Links between HP1 proteins and cancer progression.

Cancer type	HP1 protein	Altered level	Tissue/cell type/cell line	Reference
Breast	Hsalpha	Decreased	MDA-MB-231 and MCF7 breast cancer cell lines	[70]
Brain	Hsalpha	Decreased	Multiple types of embryonal brain tumors	[67]
Colon	Hsalpha	Decreased	HCT116 colon cancer cell line Caco-2 and Ht-29 colon cancer cell lines	[68,77]
Leukemia	Hsalpha, beta and gamma	Decreased	Peripheral blood leukocytes	[73,74]
Ovarian	Hsgamma	Decreased	A2780 ovarian cancer cell line	[69]
Papillary thyroid carcinoma	Hsalpha	Decreased	Papillary thyroid carcinoma tissue	[66]