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# The HP1 protein family: getting a grip on chromatin

Joel C Eissenberg* and Sarah CR Elgin†

HP1 was first described in *Drosophila* as a heterochromatin-associated protein with dosage-dependent effects on heterochromatin-induced gene silencing. Recently, membership of the HP1 protein family has expanded tremendously. A number of intriguing interactions between HP1 and other proteins have been described, implicating HP1 in gene regulation, DNA replication, and nuclear architecture.

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**Abbreviations**

| | |
|---|---|
| **GST** | glutathione *S* transferase |
| **HP1** | heterochromatin protein 1 |
| **INCENP** | inner centromere protein |
| **KRAB** | Krüppel-associated box |
| **LBR** | lamin B receptor |
| **ORC** | origin recognition complex |
| ***Pc*** | *Polycomb* |
| **PEV** | position effect variegation |
| **TIF** | transcription intermediary factor |

## Introduction

The compartmentalization of chromatin in the nuclei of higher eukaryotes has been recognized for over 100 years. At the cytological level, this is seen in the individualization of chromosomes at the onset of mitosis and in the differential condensation of heterochromatin and euchromatin in interphase nuclei [1]. At the biochemical level, the DNA of eukaryotes is packaged in nucleosomes, of which the spacing, acetylation state, and association with nonhistone proteins differs regionally.

Within euchromatic domains, gene activation is accomplished by transactivators working in concert with chromatin-remodeling complexes [2]; however, heterochromatin formation can result in the functional inactivation of regions of chromatin that would otherwise be transcriptionally active. In *Drosophila* — the organism in which heterochromatin is best-characterized — genes that become mislocalized to heterochromatin by rearrangement or transposition are silenced (reviewed in [3,4]). This silencing occurs at the transcriptional level and is correlated with a more heterochromatin-like cytological appearance in polytene chromosomes [5] and increased resistance to nuclease attack [6]. Among the nonhistone proteins primarily associated with heterochromatin, the best characterized is heterochromatin protein 1 (HP1). Our goal in this review is to summarize the structural and functional properties of HP1 family members and to highlight reported interactions with HP1 family proteins that may have functional significance.

## Identification of HP1 and its gene

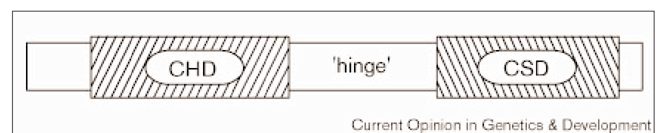
HP1 was originally identified by immunolocalization analysis of a fraction of tightly bound nonhistone chromosomal proteins prepared from *Drosophila melanogaster* embryo nuclei [7]. A monoclonal antibody was used to demonstrate a predominantly heterochromatic distribution of the protein [8] and to isolate the corresponding cDNA clone from a recombinant expression library [7]. Molecular genetic studies [9–11] determined that the gene encoding the HP1 protein was identical to a locus previously identified as a dominant suppressor of position effect variegation (PEV), *Su(var)2-5* [12,13]. PEV, a mosaic silencing, results when a euchromatic gene is placed next to or within heterochromatin. *Su(var)2-5* satisfies the genetic criteria of dosage dependency for a structural protein of heterochromatin [14]: it is a haplo-insufficient suppressor and triplo-abnormal enhancer of PEV. All known *Su(var)2-5* mutations are recessive lethal, demonstrating that HP1 is essential in *Drosophila*.

## HP1 structure and the chromo domain

The cloning of the homeotic gene silencer *Polycomb* (*Pc*) led to the identification of a sequence motif of ~44 amino acid residues shared by HP1 and PC, termed the 'chromo domain' (for chromosome organization modifier [15]). This motif has now been found in a large number of chromosomal proteins from diverse sources [16,17]. Among these are proteins from yeast, nematode, insects, chicken, frog and mammals (Table 1) that display the defining characteristics of the HP1 family: all are relatively small proteins (15–35 kDa) with an amino-terminal chromo domain and a structurally related carboxy-terminal motif, the 'chromo shadow' domain (Figure 1).

The three-dimensional structure of a chromo domain (from mouse M31) has been determined by nuclear magnetic resonance (NMR) [18]. It consists of a three-stranded

**Figure 1**




Schematic representation of the generic HP1 protein. A single amino-terminal chromo domain motif (CHD) and a single carboxy-terminal chromo shadow domain motif (CSD) are separated by a variable length linker (hinge) region. The lengths of the amino- and carboxy-terminal tails are also variable.

**Table 1**

**Members of the HP1 family.**

| Name | Organism | Size (amino acid residues) | Reported cytology | Silencing activity demonstrated | Refs |
|---|---|---|---|---|---|
| Swi6p | *S. pombe* | 328 | Centromeres, telomeres, silent mating type cassettes | + | [56] |
| Hhp1p | *T. thermophila* | 184 | Absent in micronuclei; enriched in condensed chromatin of macronuclei | – | [43] |
| pchet1 | *P. citri* | 173 | Male specific nuclear protein; not heterochromatin-specific | ND | [40] |
| pchet2 | *P. citri* | 194 | ND | ND | [40] |
| HP1 | *D. melanogaster* | 206 | Pericentric heterochromatin, telomeres, several non-pericentric sites | + | [7] |
| DvHP1 | *D. virilis* | 213 | ND | ND | [57] |
| emb\|CAB07241 | *C. elegans* | 175 | ND | ND | (a) |
| gi\|3702834 | *C. elegans* | 184 | ND | ND | (a) |
| Xhp1α | *X. laevis* | 141 | ND | ND | [37•] |
| Xhp1γ | *X. laevis* | 171 | ND | ND | [37•] |
| CHCB1 | *G. gallus* | 185 | ND | ND | [58] |
| CHCB2 | *G. gallus* | 174 | ND | ND | [58] |
| mHP1α | *M. musculus* | 191 | ND | + | [28] |
| M31; MoMOD1 | *M. musculus* | 185 | Pericentric heterochromatin | – | [59] |
| M32; MoMOD2 | *M. musculus* | 173 | Euchromatic; excluded from heterochromatin | – | [59] |
| $HP1^{hs\alpha}$ | *H. sapiens* | 191 | Pericentric heterochromatin | + | [60] |
| $HP1^{hs\beta}$ | *H. sapiens* | 185 | Pericentric heterochromatin | – | [59] |
| $HP1^{hs\gamma}$ | *H. sapiens* | 173 | Euchromatic; excluded from heterochromatin | + | [32] |

(a) GenBank database. ND, not determined.

β sheet packed against an α helix, a motif also described for two DNA-binding proteins from thermophilic archea. On the basis of its overall negative surface charge distribution, however, the chromo domain appears to be better suited for protein–protein interactions than for protein–nucleic acid interactions. Because of its high sequence homology to M31, the *Drosophila* chromo domain structure is likely to resemble the M31 chromo domain (Figure 2).

Chromo domain mutations in HP1 and PC abolish the genetic activity of these proteins [19,20]. Additionally, β-galactosidase fusion proteins with the PC chromo domain, or either the HP1 chromo or chromo shadow domains, target β-galactosidase to euchromatic PC binding sites or heterochromatic HP1 binding sites, respectively [19–21]. As expected, a chimeric HP1–PC fusion protein (in which the HP1 chromo domain is replaced with the PC chromo domain) targets β-galactosidase to both HP1 and PC binding sites. Interestingly, the chimeric protein also mislocalizes endogenous HP1 to euchromatic PC sites and endogenous PC to heterochromatin [19,22]. This latter behavior implicates the PC chromo domain and HP1 chromo shadow domain in mediating protein–protein interactions in the nucleus.

## Targets of HP1 binding in the nucleus

While there is some data supporting HP1–DNA interaction [23], much recent work has focused on identifying protein partners of HP1. Several candidates have been identified [24]. Available evidence points to roles for these proteins in gene silencing and in nuclear assembly. Interactions that implicate HP1 family members in silencing are discussed below.

### SU(VAR)3-7

*Su(var)3-7* was also identified as a dominant suppressor of heterochromatic PEV. The SU(VAR)3-7 protein includes seven zinc-finger motifs, suggesting a possible DNA binding activity. Immunofluorescent localization of SU(VAR)3-7 on larval salivary gland polytene chromosomes reveals that it has a distribution nearly identical to that of HP1, and antibodies to SU(VAR)3-7 co-immunoprecipitate HP1 from embryo extracts [25].

### SU(VAR)3-9

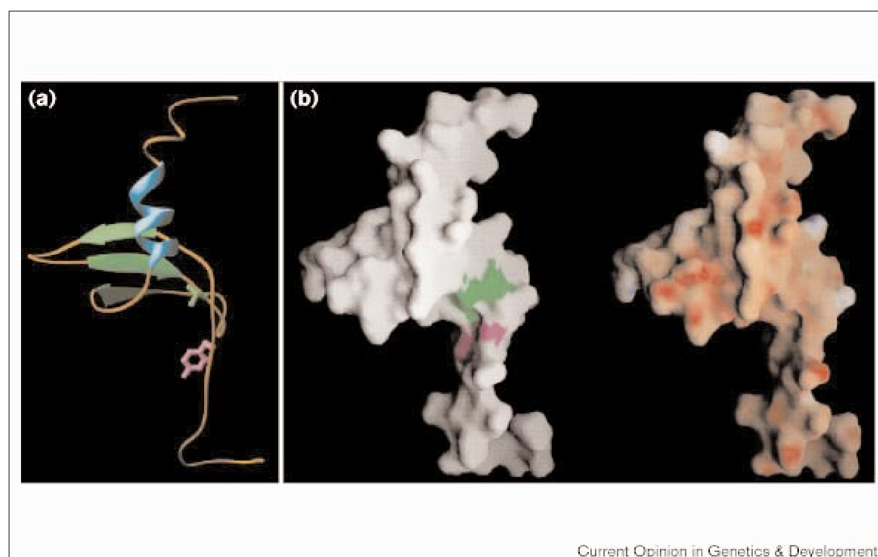
*Su(var)3-9*, another dominant suppressor of PEV, encodes a protein containing a chromo domain [26]. The protein appears to be enriched in heterochromatin [27]. A human SU(VAR)3-9 homolog, SUV39H1, can be co-immunoprecipitated from human or mouse nuclear extracts using an antibody to M31, suggesting that these proteins form a complex [27].

### TIF1α and TIF1β

The transcription intermediary factors (TIF) 1α and TIF1β interact with nuclear hormone receptors and the Krüppel-associated box (KRAB) domains of several proteins; they may function as co-activators in ligand-dependent activation of transcription and co-repressors with KRAB-containing repressor proteins. Yeast two-hybrid protein screens of a mouse embryo cDNA library using TIF1α as bait recovered clones encoding the HP1 family proteins mHP1α and mMOD1 [28].

Mutations in TIF1β gene that blocked mHP1α and mMOD1 binding *in vitro* reduce TIF1β-mediated repression of a SV40 enhancer/promoter reporter in NIH 3T3 fibroblasts [29]. TIF1β colocalizes in heterochromatin with

**Figure 2**




Three dimensional model of the *Drosophila* HP1 chromo domain. Models of the *Drosophila* chromo domain were created by using the coordinates for the MoMOD1 chromo domain [18] and substituting the *Drosophila* amino acids at positions where these sequences differ. **(a)** A ribbon model, highlighting the side chains of Tyr24 (magenta) and Val26 (green), sites of previously described mutations in *Drosophila* HP1 [19]. **(b)** Stereopair of a *Drosophila* chromo domain model, shown in space-filling representation. Left image highlights Tyr24 (magenta) and Val26 (green) and right image highlights electrostatic potential (red, negative potential; blue, positive potential). Note that both mutations occur within a groove or pocket in the chromo domain structure, suggesting a site of protein–protein interaction. Ribbon and stereopair images were rendered on a Silicon Graphics Octane unit using RIBBONS and MIDAS, respectively.

mouse M31, and in euchromatin with mouse M32 [29], consistent with an *in vivo* association between these proteins. The physiological significance of the TIF1α–mHP1α association is unclear, as the HP1 binding domain proved dispensable for TIF1α-mediated repression in a transfection assay, and no significant subnuclear colocalization of mHP1α and TIF1α has been observed [30].

## Interactions that implicate HP1 family members in nuclear assembly

### Lamin B receptor

The lamin B receptor (LBR) is an integral membrane protein of the nuclear envelope; it binds B-type lamins and double-stranded DNA, and may function as a chromatin docking site at the nuclear envelope [31]. Interaction of human LBR with the human HP1 family proteins $HP1^{hs\alpha}$ and $HP1^{hs\gamma}$ was demonstrated by affinity chromatography and by co-immunoprecipitation [32]; the interaction utilizes the chromo shadow domain [33]. *In vitro* translated HP1 binds to a purified glutathione-*S*-transferase (GST)– LBR fusion protein, indicating direct interaction [32]. Without genetic analysis, the functional significance of LBR–HP1 interaction is unclear but, in all eukaryotic cells, the inner nuclear membrane and nuclear lamina are closely associated with peripheral heterochromatin. The possibility that HP1 could promote silencing through LBR-mediated association with the nuclear membrane is intriguing, given that nuclear membrane association can promote silencing in yeast [34].

### Inner centromere protein

Inner centromere protein (INCENP), a component of the mitotic chromosome scaffold, is associated with the centromere in early metaphase but moves progressively to the spindle fibers and the plasma membrane at the presumptive cleavage furrow. The centromere-targeting amino-terminal half of INCENP interacts with $HP1^{hs\alpha}$ and $HP1^{hs\gamma}$ in a yeast two-hybrid screen of a HeLa cell cDNA library [35]. The interaction of HP1 homologs with INCENP requires the ‘hinge region’ of HP1 connecting the chromo and chromo shadow domains. The significance of the interaction remains obscure, however, as it does not seem to be required for INCENP function.

### Chromatin assembly factor 1

Chromatin assembly factor (CAF) is a three-polypeptide complex that mediates histone deposition on newly replicated DNA. A yeast two-hybrid protein screen of a mouse embryo cDNA library, using the mouse HP1 family protein MOD1 as bait, recovered cDNA clones encoding the large CAF-1 subunit p150 [36]. Comparing overlapping sequences of all cDNAs isolated in the screen, a MOD1 interacting region (MIR) was identified. MOD1 binds a GST–MIR fusion peptide *in vitro*; the MOD1 chromo shadow domain is both necessary and sufficient for this interaction.

Transient expression of GFP-tagged p150 found this fusion protein concentrated in the heterochromatin of mouse cells. Point mutations within or deletion of the DNA encoding a highly conserved hydrophobic motif of p150 reduced or eliminated p150–MOD1 interactions *in vitro* and *in vivo*. Mutations in the region of the p150 gene encoding MIR abolished heterochromatin binding of GFP–p150 fusion proteins in non-S-phase mouse L cells. Nevertheless, the mutant fusion proteins were recruited normally to replication foci during S phase, as judged their by colocalization with PCNA (proliferating cell nuclear antigen), and the fact that a p150 deletion mutant lacking the MIR functioned normally in *in vitro* nucleosome assembly. These results suggest that the HP1-binding and heterochromatin-targeting activities of the CAF-1 large subunit are dispensable for its role in nucleosome assembly during replication. Further, MOD1 synthesized in early S phase, prior to the initiation of DNA

replication, was localized to heterochromatin normally, indicating that replication-dependent chromatin assembly is not required for proper MOD1 targeting. The functional significance of CAF-1 binding in heterochromatin outside of S phase is unknown. CAF-1 may behave analogously to the satellite-binding proteins GAGA factor and PROD protein; these proteins have low affinity binding sites in certain subdomains of heterochromatin, to which they bind when excluded from their high affinity sites in euchromatin [37•].

### Origin recognition complex

Antibodies to the origin recognition complex (ORC) polypeptide ORC2 show a diffuse granular staining pattern in mitotically cycling *Drosophila* cells during interphase, with a significant concentration in the heterochromatic chromocenter [38]. During mitosis, all detectable ORC2 concentrates in the pericentric heterochromatin at a subset of HP1 binding sites. In polytene nuclei, ORC2 is distributed widely across all euchromatic chromosome arms but is largely excluded from the chromocenter.

ORC1 interacts strongly with HP1 and both the chromo and chromo shadow domains of HP1 are required for this interaction [38]. The functional significance of an Orc–HP1 interaction is unclear; while HP1 appears to be spatially restricted, Orc is thought to function at replication origins distributed throughout the genome. Orc may play a role in the establishment of heterochromatic silencing in *Drosophila*, however, analogous to the role of replication origins in silencing the silent mating type cassettes in the budding yeast *Saccharomyces cerevisiae*. Here, Orc appears to act as a platform to recruit the silencing protein Sir1p [39]. As in *Drosophila*, there must be more to the mechanism, as replication origins are widely dispersed throughout the chromosomes, whereas silencing in yeast is restricted to the silent mating type cassettes, telomeres and rDNA.

### Self-association

HP1 family proteins also undergo self-association, reported for *P. citri* HP1 proteins [40], $HP1^{hs\alpha}$ and mHP1α [28,33]; heterologous interactions between $HP1^{hs\alpha}$ and $HP1^{hs\gamma}$ have also been observed [33]. In the case of the human proteins, the associations depend on the chromo shadow domain.

Thus, there appears to be a complex and potentially dynamic collection of HP1-dependent interactions occurring in a variety of eukaryotic cells. Where the interactions have been mapped, most involve the chromo shadow domain. It seems unlikely that such a relatively small domain could accommodate simultaneously such a wide range of partners. Instead, distinct populations of HP1 may be involved in distinct interactions. Differential HP1 phosphorylation could regulate these interactions.

## Phosphorylation of HP1 and the regulation of heterochromatin assembly

In *Drosophila*, HP1 is multiply phosphorylated by serine/threonine kinases, one of which is casein kinase II (CKII) [41•]; CKII phosphorylation of HP1 is required for efficient heterochromatin targeting [41•]. There is also indirect evidence for tyrosine phosphorylation of HP1 [19]. Biochemical fractionation of HP1 suggests that differential HP1 phosphorylation may be associated with distinct complexes [42]. Human and *Tetrahymena* HP1 proteins are also differentially phosphorylated; hyperphosphorylation of the *Tetrahymena* Hhp1p is induced by starvation and is correlated with decreased nuclear volume [43]. In humans, hyperphosphorylation of $HP1^{hs\alpha}$ and $HP1^{hs\gamma}$ is correlated with mitosis [44•]. The dynamic nature of HP1 phosphorylation suggests a regulatory function for this process, although further genetic and biochemical studies are needed.

## Mechanism of HP1-mediated silencing

Although genetic and cytological evidence in *Drosophila* clearly implicates HP1 in heterochromatic position effect silencing, both the mechanism of silencing and the role of HP1 in the mechanism remain unknown. The structure of the HP1 protein, with two related heterochromatin-targeting domains, suggests that it acts as a bifunctional cross-linker, perhaps organizing higher order chromatin structure by linking or anchoring chromatin subunits.

In chromosome rearrangements that place euchromatic genes next to a heterochromatic breakpoint, HP1 becomes visibly associated with euchromatic regions in *cis* across the breakpoint [45]. This physical association correlates with the silencing of adjacent genes; however, a cytological association of HP1 with regions silenced by PEV is apparently not obligatory, as a similar immunostaining experiment found no detectable HP1 staining at a copy of the *brown* locus silenced in *trans* by $brown^{Dominant}$ [46].

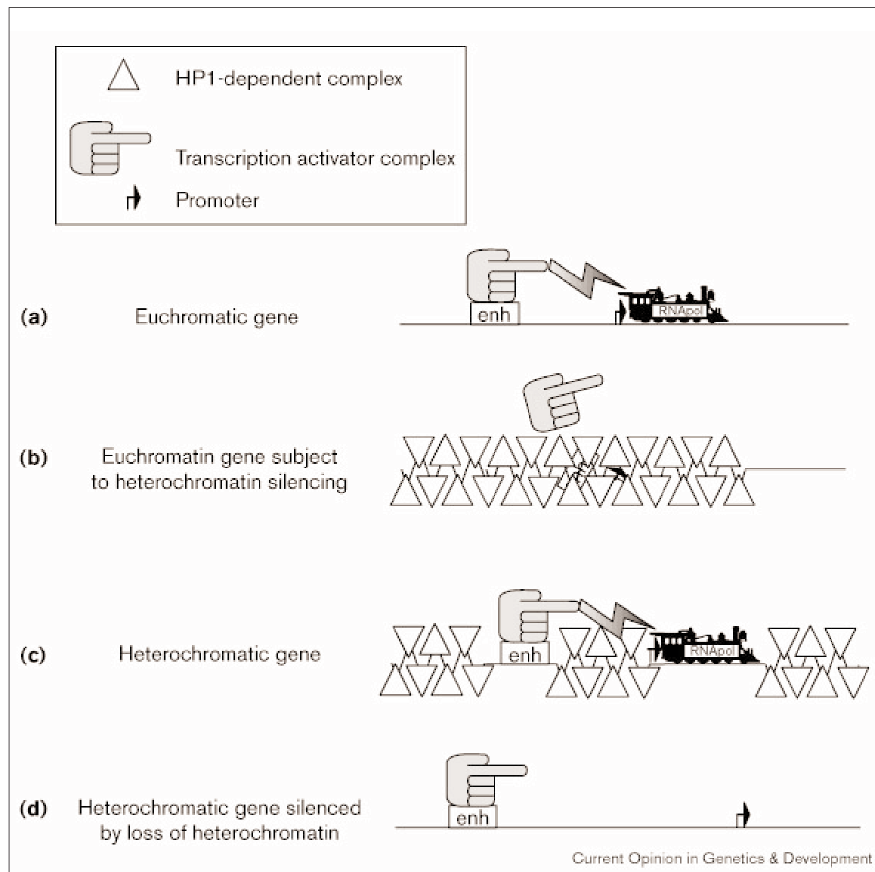
Arrays of *P* transposons carrying a mini-*white* gene result in silencing of the *white* marker [47]. HP1 is recruited to these silenced arrays in polytene chromosomes [48•]. Interestingly, lower amounts of HP1 are also detectable at single copy nonsilenced *white* transgenes and at transposon arrays carrying a different eye color marker that do not exhibit silencing. Apparently, the presence of HP1 alone is not sufficient to establish silencing; perhaps some threshold of HP1 concentration is required.

At the biochemical level, heterochromatin silencing is correlated with reduced accessibility of promoter sequences to nuclease attack [6], while suppression of PEV by HP1 mutation is correlated with increased accessibility [49]. The mechanism by which HP1 mediates this differential accessibility is unknown. High resolution footprinting analysis suggests a loss of transcription factor and paused RNA polymerase from the silenced heat shock promoters [50••].

## What does HP1 do normally?

In *Drosophila*, examples of HP1-dependent silencing involve chromosome rearrangements or transposon insertions; however, the heterochromatin of flies does include a number of

**Figure 3**




Cartoon representation of the role of an HP1-dependent complex as a chromatin organizer, promoting silencing of euchromatic genes and expression of heterochromatic genes in heterochromatic domains. **(a)** In euchromatin, a euchromatic enhancer (enh) is bound by an activator complex, which acts to recruit RNA polymerase (shown as a train) to the promoter. **(b)** When a euchromatic gene lies next to a heterochromatic breakpoint, HP1-dependent complexes spread across the breakpoint to assemble heterochromatin over euchromatic sequences, making the template inaccessible to activators. **(c)** HP1-dependent complexes act to organize heterochromatic DNA so as to accommodate binding of heterochromatic gene activators and their target promoters. **(d)** When a heterochromatic locus is mislocalized to euchromatin (or mutations interfere with HP1-dependent complex formation), the normal HP1-mediated organization of the locus is lost, and the gene is silenced.

genes, some of which have been shown to require a heterochromatin context for their normal expression [51,52]. In rearrangements that separate the genes from their flanking heterochromatin such genes are misregulated. Mutations in several loci that cause suppression of classical PEV enhance the misregulation of rearranged heterochromatic genes [53]; these mutations include alleles of *Su(var)2-5*, the locus encoding HP1. In addition, certain pairwise combinations of PEV modifiers including *Su(var)2-5* result in misregulation of the heterochromatin gene *light*, when *light* remains in its normal chromosomal position [54•].

A second role for HP1 is suggested by the report that the metaphase chromosomes in HP1 mutant larval neuroblasts show a high frequency of telomere associations [55]. As HP1 is found at telomeres, the telomere fusions that occur in larvae lacking zygotic HP1 suggest that HP1 might function to protect telomeres; however, HP1 might function in the pericentric heterochromatin to regulate *trans*-acting modifiers of telomeres, such as Het-A elements, which are responsible for telomere stabilization.

Normally, HP1 may simply function as an organizer of higher order chromatin structure in the nucleus (Figure 3). This organizing property could serve to accommodate the transcription of genes that normally reside within heterochromatin. When the normal organization of heterochromatin is lost — through rearrangement to euchromatin or by depletion of structural subunits — misregulation of heterochromatic genes would result. Conversely, rearrangements that place euchromatic genes next to a heterochromatic breakpoint could make genes near the breakpoint vulnerable to assembly into HP1-dependent heterochromatin, silencing those genes by template occlusion.

## Conclusions

The HP1 family of proteins represents the best-characterized heterochromatin-associated nonhistone chromosomal protein family in the eukaryotic kingdom. Its remarkable evolutionary conservation suggests a fundamental role for HP1 proteins in nuclear organization and a highly conserved set of macromolecular interactions. The role of HP1 proteins in mediating position-effect silencing has proven especially useful in genetic strategies aimed at identifying candidate partners for HP1 in heterochromatin assembly. Future work on HP1 and its partners will be directed at defining its role as a subunit of heterochromatin, as a cofactor in gene regulation, and as an essential player in the dynamic organization of nuclear architecture. Key to the achievement of these goals will be the application of genetic assays to test the functional significance of cytological and biochemical correlations.

## Update

Since the submission of this review, two new reports have appeared that significantly extend our understanding of HP1 family activities. Festenstein *et al.* [61••] report the first study to show that a mammalian HP1 family member has dosage dependent effects on heterochromatin silencing in transgenic mice. Smothers and Henikoff [62•] identify a consensus chromo shadow domain binding motif that may underlie some HP1-dependent interactions, as well as HP1 self-association.

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## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Heitz E: **Das Heterochromatin der Moose.** *Jb Wiss Bot* 1928, **69**:728-818. [Title translation: The heterochromatin of moss.]
2. Workman JL, Kingston RE: **Alteration of nucleosome structure as a mechanism of transcriptional regulation.** *Annu Rev Biochem* 1998, **67**:545-579.
3. Spofford JB: **Position-effect variegation in *Drosophila*.** In *Genetics and Biology of Drosophila*, vol 1c. Edited by Ashburner M, Novitski E. London: Academic Press; 1976:955-1019.
4. Weiler KS, Wakimoto BT: **Heterochromatin and gene expression in *Drosophila*.** *Annu Rev Genet* 1995, **29**:557-605.
5. Zhimulev IF: **Morphology and structure of polytene chromosomes.** *Adv Genet* 1996, **34**:1-147.
6. Wallrath LL, Elgin SCR: **Position effect variegation in *Drosophila* is associated with an altered chromatin structure.** *Genes Dev* 1995, **9**:1263-1277.
7. James TC, Elgin SCR: **Identification of a nonhistone chromosomal protein associated with heterochromatin in *Drosophila* and its gene.** *Mol Cell Biol* 1986, **6**:3862-3872.
8. James TC, Eissenberg JC, Craig C, Dietrich V, Hobson A, Elgin SCR: **Distribution patterns of HP1, a heterochromatin-associated nonhistone chromosomal protein of *Drosophila*.** *Eur J Cell Biol* 1989, **50**:170-180.
9. Eissenberg JC, James TC, Foster-Hartnett DM, Hartnett T, Ngan V, Elgin SCR: **Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*.** *Proc Natl Acad Sci USA* 1990, **87**:9923-9927.
10. Eissenberg JC, Morris GD, Reuter G, Hartnett T: **The heterochromatin-associated protein HP-1 is an essential protein in *Drosophila* with dosage-dependent effects on position-effect variegation.** *Genetics* 1992, **131**:345-352.
11. Eissenberg JC, Hartnett T: **A heat shock-activated cDNA rescues the recessive lethality of mutations in the heterochromatin-associated protein HP1 of *Drosophila melanogaster*.** *Mol Gen Genet* 1993, **240**:333-338.
12. Sinclair DAR, Mottus RC, Grigliatti TA: **Genes which suppress position-effect variegation in *Drosophila melanogaster* are clustered.** *Mol Gen Genet* 1983, **191**:326-333.
13. Wustmann G, Szidonya J, Taubert H, Reuter G: **The genetics of position-effect variegation modifying loci in *Drosophila melanogaster*.** *Mol Gen Genet* 1989, **217**:520-527.
14. Locke J, Kotarski MA, Tartof KD: **Dosage dependent modifiers of position effect variegation in *Drosophila* and a mass action model that explains their effect.** *Genetics* 1988, **120**:181-198.
15. Paro R, Hogness D: **The polycomb protein shares a homologous domain with a heterochromatin associated protein of *Drosophila*.** *Proc Natl Acad Sci USA* 1991, **88**:263-267.
16. Aasland R, Stewart AF: **The chromo shadow domain, a second chromo domain in heterochromatin-binding protein 1, HP1.** *Nucleic Acids Res* 1995, **23**:3163-3173.
17. Koonin EV, Zhou SB, Lucchesi JC: **The chromo superfamily — new members, duplication of the chromo domain and possible role in delivering transcription regulators to chromatin.** *Nucleic Acids Res* 1995, **23**:4229-4233.
18. Ball LJ, Murzina NV, Broadhurst RW, Raine ARC, Archer SJ, Stott FJ, Murzin AG, Singh PB, Domaille PJ, Laue ED: **Structure of the chromatin binding (chromo) domain from mouse modifier protein 1.** *EMBO J* 1997, **16**:2473-2481.
19. Platero JS, Hartnett T, Eissenberg JC: **Functional analysis of the chromo domain of HP1.** *EMBO J* 1995, **14**:3977-3986.
20. Messmer S, Franke A, Paro R: **Analysis of the functional role of the Polycomb chromo domain in *Drosophila melanogaster*.** *Genes Dev* 1992, **6**:1241-1254.
21. Powers J, Eissenberg JC: **Overlapping domains of the heterochromatin associated protein HP1 mediate nuclear localization and heterochromatin binding.** *J Cell Biol* 1993, **120**:291-299.
22. Platero JS, Sharp EJ, Adler PN, Eissenberg JC: ***In vivo* assay for protein–protein interactions using *Drosophila* chromosomes.** *Chromosoma* 1996, **104**:393-404.
23. Sugimoto K, Yamada T, Muro Y, Himeno M: **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* 1996, **120**:153-159.
24. Wallrath LL: **Unfolding the mysteries of heterochromatin.** *Curr Opin Genet Dev* 1998, **8**:147-153.
25. Cléard F, Delattre M, Spierer P: **SU(VAR)3-7, a *Drosophila* heterochromatin-associated protein and companion of HP1 in the genomic silencing of position-effect variegation.** *EMBO J* 1997, **16**:5280-5288.
26. Tschiersch B, Hofmann A, Krauss V, Dorn R, Korge G, Reuter G: **The protein encoded by the *Drosophila* position-effect variegation suppressor gene *Su(var)3-9* combines domains of antagonistic regulators of homeotic gene complexes.** *EMBO J* 1994, **13**:3822-3831.
27. Aagaard L, Laible G, Selenko P, Schmid M, Dorn R, Schotta G, Kuhfittig S, Wolf A, Lebersorger A, Singh PB *et al.*: **Functional mammalian homologues of the *Drosophila* PEV-modifier *Su(var)3-9* encode centromere-associated proteins which complex with the heterochromatin component M31.** *EMBO J* 1999, **18**:1923-1938.
28. Le Douarin B, Nielsen AL, Garnier JM, Ichinose H, Jeanmougin F, Losson R, Chambon P: **A possible involvement of TIF1α and TIF1β in the epigenetic control of transcription by nuclear receptors.** *EMBO J* 1996, **15**:6701-6715.
29. Ryan RF, Schultz DC, Ayyanathan K, Singh PB, Friedman JR, Fredericks WJ, Rauscher III FJ: **KAP-1 corepressor protein interacts and colocalizes with heterochromatic and euchromatic HP1 proteins: a potential role for Krüppel-associated box-zinc finger proteins in heterochromatin-mediated gene silencing.** *Mol Cell Biol* 1999, **19**:4366-4378.
30. Remboutsika E, Lutz Y, Gansmuller A, Vonesch JL, Losson R, Chambon P: **The putative nuclear receptor mediator TIF1α is tightly associated with euchromatin.** *J Cell Sci* 1999, **112**:1671-1683.
31. Pyrpasopoulou A, Meier J, Maison C, Simos G, Georgatos SD: **The lamin B receptor (LBR) provides essential chromatin docking sites at the nuclear envelope.** *EMBO J* 1996, **15**:7108-7119.
32. Ye Q, Worman HJ: **Interaction between an integral protein of the muclear envelope inner membrane and human chromodomain proteins homologous to *Drosophila* HP1.** *J Biol Chem* 1996, **271**:14653-14656.
33. Ye Q, Callebaut I, Pezhman A, Courvalin JC, Worman HJ: **Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR.** *J Biol Chem* 1997, **272**:14983-14989.
34. Andrulis ED, Neiman AM, Zappulla DC, Sternglanz R: **Perinuclear localization of chromatin facilitates transcriptional silencing.** *Nature* 1998, **394**:592-595.

35. Ainsztein AM, Kandels-Lewis SE, Mackay AM, Earnshaw WC: **INCENP centromere and spindle targeting: identification of essential conserved motifs and involvement of heterochromatin protein HP1.** *J Cell Biol* 1998, **143**:1763-1774.

36. Murzina N, Verreault A, Laue E, Stillman B: **Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins.** *Mol Cell* 1999, **4**:529-540.

37. Platero JS, Csink AK, Quintanilla A, Henikoff S: **Changes in**
• **chromosomal localization of heterochromatin-binding proteins during the cell cycle in *Drosophila*.** *J Cell Biol* 1998, **140**:1297-1306.
The *Drosophila* chromosomal proteins GAGA factor and PROD are both heterochromatin-associated in metaphase chromosomes but are absent from heterochromatin in interphase polytene tissues. The authors present data indicating that both proteins have high-affinity binding sites in euchromatin and low-affinity binding sites in specific heterochromatin-imbedded satellite sequences. Protein redistribution from euchromatin to heterochromatin may be driven by mitotic chromosome condensation. Importantly, the authors observe that heterochromatin binding is absent in *Drosophila* species lacking particular satellites. The results suggest that caution should be used in assigning functional significance to the simple observation of heterochromatin binding.

38. Pak DTS, Pflumm M, Chesnodov I, Huang DW, Kellum R, Marr J, Romanowski P, Botchan MR: **Association of the origin recognition complex with heterochromatin and HP1 in higher eukaryotes.** *Cell* 1997, **91**:311-323.

39. Fox CA, Ehrenhofer-Murray AE, Loo S, Rine J: **The origin recognition complex, SIR1, and the S phase requirement for silencing.** *Science* 1997, **276**:1547-1551.

40. Epstein H, James TC, Singh PB: **Cloning and expression of *Drosophila* HP1 homologs from a mealybug, *Planococcus citri*.** *J Cell Sci* 1992, **101**:463-474.

41. Zhao T, Eissenberg JC: **Phosphorylation of heterochromatin**
• **protein 1 by casein kinase II is required for efficient heterochromatin binding in *Drosophila*.** *J Biol Chem* 1999, **274**:15095-15100.
This group previously showed that HP1 is multiply phosphorylated and that hyperphosphorylation is correlated with heterochromatin assembly. In this paper, they report that three phosphorylation sites are targets for casein kinase II (CKII). Alanine substitution mutations that block CKII phosphorylation interfere with efficient heterochromatin targeting by HP1.

42. Huang DW, Fanti L, Pak DT, Botchan MR, Pimpinelli S, Kellum R: **Distinct cytoplasmic and nuclear fractions of *Drosophila* heterochromatin protein 1: their phosphorylation levels and associations with origin recognition complex proteins.** *J Cell Biol* 1998, **142**:307-318.

43. Huang H, Smothers JF, Wiley EA, Allis CD: **A nonessential HP1-like protein affects starvation-induced assembly of condensed chromatin and gene expression in macronuclei of *Tetrahymena thermophila*.** *Mol Cell Biol* 1999, **19**:3624-3634.

44. Minc E, Allory Y, Courvalin JC, Worman HJ, Buendia B: **Localization**
• **and phosphorylation of HP1 proteins during the cell cycle in mammalian cells.** *Chromosoma* 1999, **108**:220-234.
This paper reports a detailed analysis of the nuclear distribution of the three known human HP1 family members during the cell cycle. Two of the three are heterochromatin-associated, while the third is dispersed at numerous euchromatic sites. The authors also show that two of the three are phosphorylated, with more extensive phosphorylation associated with M phase in the cell cycle.

45. Belyaeva ES, Demakova OV, Umbetova GH, Zhimulev IF: **Cytogenetic and molecular aspects of position-effect variegation in *Drosophila melanogaster*. V. Heterochromatin-associated protein HP1 appears in euchromatic chromosomal regions that are inactivated as a result of position-effect variegation.** *Chromosoma* 1993, **102**:583-590.

46. Belyaeva ES, Koryakov DE, Pokholkova GV, Demakova OV, Zhimulev IF: **Cytological study of the *brown* dominant position effect.** *Chromosoma* 1997, **106**:124-132.

47. Dorer DR, Henikoff S: **Expansions of transgene repeats cause heterochromatin formation and gene silencing in *Drosophila*.** *Cell* 1994, **77**:993-1002.

48. Fanti L, Dorer DR, Berloco M, Henikoff S, Pimpinelli S:
• **Heterochromatin protein 1 binds transgene arrays.** *Chromosoma* 1999, **107**:286-292.
A previous study demonstrated that arrays of *P*-elements containing a *white* transgene resulted in variegated silencing of *white*. Silencing appeared to be a result of the assembly of ectopic heterochromatin at the array locus. This paper shows that the silenced arrays recruit HP1. Interestingly, a single copy *white* transgene, which shows no variegation, still binds HP1, suggesting that HP1-dependent silencing might require threshold quantities of HP1.

49. Cryderman DE, Cuaycong MH, Elgin SCR, Wallrath LL: **Characterization of sequences associated with position-effect variegation at pericentric sites in *Drosophila* heterochromatin.** *Chromosoma* 1998, **107**:277-285.

50. Cryderman DE, Tang HB, Bell C, Gilmour DS, Wallrath LL: **Hetero-**
•• **chromatic silencing of *Drosophila* heat shock genes acts at the level of promoter potentiation.** *Nucleic Acids Res* 1999, **27**:3364-3370.
Previous work has shown that regions subject to heterochromatic silencing are less accessible to nuclease attack, suggesting that heterochromatinization might reduce transcription factor access as well. This paper provides the first evidence that binding of three transcription factors – GAGA factor, TFIID and RNA polymerase II – is reduced at a silenced locus.

51. Wakimoto B, Hearn M: **The effects of chromosome rearrangements on the expression of heterochromatic genes in chromosome 2L of *Drosophila melanogaster*.** *Genetics* 1990, **125**:141-154.

52. Eberl DF, Duyf BJ, Hilliker AJ: **The role of heterochromatin in the expression of a heterochromatic gene, the *rolled* locus of *Drosophila melanogaster*.** *Genetics* 1993, **134**:277-292.

53. Hearn MG, Hedrick A, Grigliatti TA, Wakimoto BT: **The effect of modifiers of position-effect variegation on the variegation of heterochromatic genes of *Drosophila melanogaster*.** *Genetics* 1991, **128**:785-797.

54. Clegg NJ, Honda BM, Whitehead IP, Grigliatti TA, Wakimoto B,
• Brock HW, Lloyd VK, Sinclair DAR: **Suppressors of position-effect variegation in *Drosophila melanogaster* affect expression of the heterochromatic gene *light* in the absence of a chromosome rearrangement.** *Genome* 1998, **41**:495-503.
Dominant suppressors of PEV were previously shown to enhance the variegation of the heterochromatic gene *light* when it is moved to euchromatin by a chromosome rearrangement. This paper shows that certain combinations of dominant PEV suppressors (including combinations with a mutant allele of *Su*[*var*]*2-5*) cause misregulation of *light* with this heterochromatic gene in its normal chromosomal context. This result suggests that the products of such PEV suppressors, including HP1, are required normally for the proper regulation of heterochromatic genes.

55. Fanti L, Giovinazzo G, Berloco M, Pimpinelli S: **The heterochromatin protein 1 prevents telomere fusions in *Drosophila*.** *Mol Cell* 1998, **2**:527-538.

56. Lorentz A, Ostermann K, Fleck O, Schmidt H: **Switching gene *swi6*, involved in repression of silent mating-type loci in fission yeast, encodes a homologue of chromatin-associated proteins from *Drosophila* and mammals.** *Gene* 1994, **143**:139-143.

57. Clark RF, Elgin SCR: **Heterochromatin protein 1, a known suppressor of position-effect variegation, is highly conserved in *Drosophila*.** *Nucleic Acids Res* 1992, **20**:6067-6074.

58. Yamaguchi K, Hidema S, Mizuno S: **Chicken chromobox proteins: cDNA cloning of CHCB1, -2, -3 and their relation to W-heterochromatin.** *Exp Cell Res* 1998, **242**:303-314.

59. Singh PB, Miller JR, Pearce J, Kothary R, Burton RD, Paro R, James TC, Gaunt SJ: **A sequence motif found in a *Drosophila* heterochromatin protein is conserved in animals and plants.** *Nucleic Acids Res* 1991, **19**:789-794.

60. Saunders WS, Chue C, Goebl M, Craig C, Clark RF, Powers JA, Eissenberg JC, Elgin SCR, Rothfield NF, Earnshaw WC: **Molecular cloning of a human homologue of Drosophila heterochromatin protein HP1 using anticentromere autoantibodies with anti-chromo specificity.** *J Cell Sci* 1993, **104**:573-582

61. Festenstein R, Sharghi-Namini S, Fox M, Roderick K, Tolaini M, Norton T,
•• Saveliev A, Kioussis D, Singh P: **Heterochromatin protein 1 modifies mammalian PEV in a dose- and chromosomal-context-dependent manner.** *Nat Genet* 1999, **23**:457-461.
The authors exploit previously established transgenic mouse lines in which a human D2 locus control region mutation construct was found to variegate when inserted in heterochromatin. This paper shows that the variegation is enhanced by overexpression of mouse M31. This is the first demonstration of dosage-dependent silencing by an HP1 family protein in a metazoan other than *Drosophila*.

62. Smothers JF, Henikoff S: **The HP1 chromo shadow domain binds a**
• **consensus peptide pentamer.** *Curr Biol* 2000, **10**:27-30.
By probing a random phage display library with a *Drosophila* HP1 chromo shadow domain, the authors identify a consensus peptide motif sufficient for chromo shadow domain binding. Sequences highly related to this consensus can be found in some reported HP1 family-interacting proteins, as well as in the HP1 chromo shadow domain itself. Importantly, self-dimerization of the chromo shadow domain *in vitro* is disrupted by a peptide based on the consensus motif.