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Review

Heterochromatin protein 1: a pervasive controlling influence

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Abstract. Heterochromatin protein 1 (HP1), a component of condensed chromatin, was discovered more than 10 years ago and subsequently found to play important roles in chromosomal biology and gene silencing. Consistent with the hypothesis that post-translational modifications of histones may functionally 'mark' DNA sequences, HP1 was found to bind to 'silent' chromatin via the methylated lysine 9 (K9) residue on the histone H3 tail that protrudes from the nucleosome. The discovery of several HP1-associating proteins has given us insight into how HP1 may function. Although initially found to localise predominantly at heterochromatin, recent data suggest that HP1 also localises and dynamically participates in gene regulation in euchromatin. Moreover, the initial definition of HP1 as a gene repressor may need to be revisited, as HP1 has been shown, in some cases, to localise at transcriptionally active chromosomal sites. Here we review current knowledge on HP1 and explore possible mechanisms whereby HP1 might exert divergent effects on gene regulation.

Key words. HP1; PEV; position effect variegation; chromatin; heterochromatin protein 1; heterochromatin; epigenetics; histone modification; gene silencing; gene regulation; chromatin modifiers; epigenetic modifiers; histone; histone code.

Introduction

In the late 1920s, Emil Heitz [1] introduced the term 'heterochromatin' to describe a form of chromatin that remained condensed throughout the cell cycle in contrast to the rest of the genome (euchromatin) that underwent extensive decondensation during the transition from metaphase to interphase. Though its name has a purely cytological origin, heterochromatin exhibits distinct genetic and biochemical properties from euchromatin. It consists mainly of repetitive DNA sequences, contains hardly any transcriptionally active/competent genes and in many cases replicates late in S-phase. In fission yeast and higher eukaryotes, such a 'permanently' condensed chromatin structure is consistently associated with pericentromeric and telomeric regions of chromosomes. The first clue that this condensed form of chromatin influences gene activity came a few years after Heitz's observation. In 1930, Hermann Muller described *Drosophila* mutants generated by X-ray irradiation that showed variegated (mosaic) expression of the *white* gene in the eyes [2]. This stochastic silencing of the *white* gene, whose expression in every eye cell in normal flies leads to uniform red pigmentation of the eyes, was found to correlate with a chromosomal translocation of the *white* gene from its normal euchromatic location to the vicinity of centromeric heterochromatin and was termed position effect variegation (PEV). The detailed molecular mechanisms for PEV are not known, but whether in *cis*- or *trans*- or both, the neighbouring 'heterochromatic structure' ap-

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pears to be stochastically spreading into the variegating gene. Since the silencing effect seen in PEV seems to be stable through successive cell divisions, resulting in red and white patches in the mature *Drosophila* eye, heterochromatin has become the focus of intense study to understand mechanisms involved in heritable gene expression/repression [3, 4].

The major leap in our understanding of heterochromatin or heterochromatin-mediated gene silencing did not happen until the 1980s, when extensive mutagenesis analysis of modifiers of PEV in *Drosophila* was carried out. This resulted in the identification of more than 100 genes that either enhanced or suppressed PEV when they were mutated. Heterochromatin protein 1 (HP1) was found to be a dominant suppressor of PEV and had been previously characterised as a non-histone component of chromatin that predominantly localises to pericentromeric heterochromatin [5, 6]. In *Drosophila* and mammalian models of PEV, overexpression of HP1 often enhances silencing of variegating genes [7, 8]. On the other hand, heterozygous mutation of HP1 results in less silencing of variegating genes [9]. HP1 is likely to be essential for normal development of eukaryotic organisms since homozygous mutation of the gene that encodes *Drosophila* HP1, *Su(var)2-5*, leads to lethality at the third instar larval stage [10, 11], when the maternal supply of HP1 proteins is normally drastically reduced.

The localisation and roles of HP1 at heterochromatic regions have been relatively well elucidated. However, it is clear that HP1 not only localises to heterochromatic regions but also to euchromatic regions. In addition to the DNA repeats found at centromeres and telomeres, repetitive DNA elements scattered within euchromatic regions are also associated with heterochromatin formation. In contrast, some studies have suggested that HP1 is involved in more refined silencing of single-copy genes in euchromatic regions [12–14]. In addition, it was recently shown that HP1 is associated with transcriptionally active domains of polytene chromosomes [15]. Hence, despite its name and predominant localisation at heterochromatin, HP1 seems to exhibit different roles in different nuclear environments. Here we review our current understanding of various aspects of HP1, including its structure, localisation and function, and discuss the diverse mechanisms HP1 may be adopting in gene regulation.

Structure

HP1 is highly conserved through evolution from *Schizosaccharomyces pombe* to human. It was first identified in *Drosophila* (HP1/HP1a, and recently identified HP1b and HP1c [16]), and subsequently homologues of HP1 were discovered in *S. pombe* (Swi6), *Xenopus* (Xhp1α and Xhp1γ), chicken (CHCB1, CHCB2 and CHCB3), mouse (HP1α, HP1β and HP1γ) and human ($HP1^{Hs}\alpha$, $HP1^{Hs}\beta$ and $HP1^{Hs}\gamma$) [16, 17]. Each HP1 molecule has an evolutionarily conserved N-terminal chromo domain (CD) and C-terminal chromo-shadow domain (CSD) [18]. Three-dimensional (3D) structures of CD and CSD have been resolved by nuclear magnetic resonance (NMR) spectroscopy [19, 20] and X-ray crystallography [21]. Both CD and CSD are globular domains consisting of an anti-parallel, three-stranded β-sheet, against which one or two α-helices are packed, respectively. Although CD and CSD have very similar domain structures, they seem to serve distinct functions. CD is known to be the chromatin-binding domain [22–27]. Lack of, or mutations in, this domain abolish localisation of HP1 at centromeric heterochromatin [28]. Mechanisms for HP1 interaction with chromatin and those for its predominant localisation at pericentromeric heterochromatin had been unclear until the gene product of the suppressor of variegation *su(var)3-9*, another dominant suppresser of PEV, was characterised [29].

Like HP1, the gene product of *su(var)3-9* is highly conserved through evolution. Thus, SUV3-9 (*Drosophila*) [30], SUV39H1/2 (human), Suv39h1/2 (mouse) [29], Clr4 (yeast) [31] histone methyltransferases (HMTases) possess an HMT activity specific for the lysine 9 (K9) residue on the histone H3 N-terminus tail. [24, 32]. HP1 interacts with chromatin by binding to methylated K9 on histone H3 (methyl H3K9) through the CD [22–27]. Such modification of histone H3 is highly abundant in pericentromeric heterochromatin; thus HP1 is preferentially targeted to those regions. Consistent with these findings, interaction between HP1 and chromatin can be disrupted by preventing H3K9 methylation by acetylating the K9 residue using a potent histone deacetylase (HDAC) inhibitor, trichostatin A (TSA) [33], or by mutating *Suv39h* [28]. Analysis by NMR and X-ray crystallography revealed that the specific binding of CD to methyl H3K9 is mediated by three key hydrophobic side chains that form the 'hydrophobic box', while other specific sites of the CD interact with the neighbouring H3 amino acid residues to structure the flexible N-terminal tail [25–27]. The latter point may reflect the fact that some modifications in neighbouring residues affect HP1 binding. Moreover, the CD has also been shown to bind to the C-terminus globular domain of histone H3 [34]. The structural analysis indicated that the interaction between CD and methyl H3K9 peptide is rather weak, despite the fact that HP1 shows high affinity binding to chromatin in salt extraction assays on nuclear extract. Thus, in the in vivo situation, stable HP1-chromatin interaction may be mediated by binding of HP1 to the core globular domain of the histone and/or interaction with HP1-associating proteins and RNA following the CD-methyl H3K9 interaction [35–37]. In contrast to the CD, the

CSD has been regarded as the dimerisation domain [20]. In solution, CSDs readily dimerise, whereas CDs remain monomeric. Thus, two HP1 molecules can interact with each other to form a homo- or heterodimer among or between different HP1 isoforms. Moreover, the CSD is often involved in protein-protein interactions with HP1-associating proteins [20, 21]. Phage display experiments were used to screen for consensus peptides that bind to the CD and CSD domains of HP1 [38]. No peptide that bound strongly to CD was identified, possibly because the peptides expressed on the surface of bacteriophages are not post-translationally modified. In contrast, the CSD bound tightly to peptides that are related to the consensus PxVxL (where P = Proline, V = Valine, L = Leucine and x is any amino acid). This peptide binds as a monomer into a hydrophobic pocket that is contributed by the two subunits of a CSD dimer [37].

Proteins such as transcriptional intermediary factors (TIFs) [20, 39–42], Su(z)12 [43], TATA binding protein (TBP)-associated factor of 130 kD ($TAF_{II}130$) [44] and the p150 large subunit of chromatin assembly factor 1 (CAF1) [20, 42, 45], NIPBL [46], contain this consensus sequence, and dimerisation of CSD seems to be required for interaction of these proteins with HP1 [20]. Moreover, some proteins such as ATRX, lamin B receptor (LBR) and Sp100a that contain variant PxVxL motifs are shown to bind to HP1 in vitro [46]. However, not all proteins known to directly interact with HP1 possess this consensus sequence. In addition, it has been reported that some proteins interact with HP1 through the CD or hinge [47]. For example, *Drosophila* HP1 has been described to interact with origin recognition complexes (ORCs) via the CD [48]. Several components of the nuclear envelope, such as lamina-associated polypeptide 2β (LAP2β) and lamin B, are known to bind to the CD of murine HP1β by binding assay [49]. It is likely, therefore, that the CSD is not the only domain mediating protein-protein interaction.

These key globular domains, the CD and the CSD, are joined by a less conserved, flexible hinge region. Although the CD and the CSD of mammalian HP1 isoforms share around 50–70 % identity, the hinge region shows only 25–30 % identity with *Drosophila* HP1 [47]. Unlike the CD or the CSD, not many proteins are known to interact with HP1 through the hinge region, but the binding of human HP1α with the inner centromere protein (INCENP) seems to require the hinge region [50]. Furthermore, RNA and chromatin binding activity of the hinge region has been suggested [36, 51]. As well as the interaction between the CD and methyl H3K9, such activity has been shown to be necessary to target the HP1 molecule to pericentric domains [36]. Another interesting feature of the hinge region is that it contains a number of consensus phosphorylation sites for various kinases [52]. Mutation of phosphorylation sites that mimic a hyper-phosphorylated state of *Drosophila* HP1 enhanced self-dimerisation and methyl H3K9 binding activity and abolished the ability of HP1 to interact with ORC1 and HP1/ORC-associated protein (HOAP) [53]. On the other hand, mutation that incapacitates phosphorylation at the hinge disturbed HP1 binding to another member of the ORCs, ORC2. Such observations give rise to the possibility that phosphorylation of HP1 at the hinge may play important roles in regulating the activity of the CD and the CSD or the HP1 molecule as a whole.

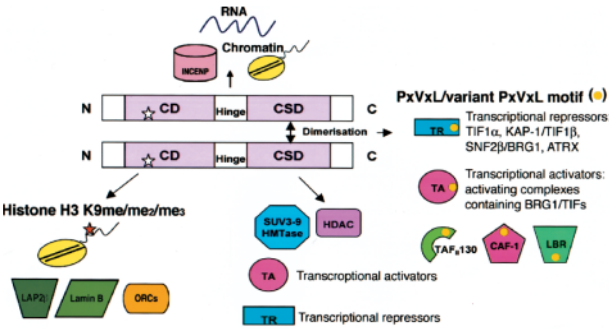

Figure 1. A schematic diagram of HP1. All HP1 isoforms (HP1α/β/γ) have a conserved N-terminus chromo domain (CD) and a C-terminus chromo-shadow (CSD) domain. These domains are joined by a flexible hinge. The CD is involved in interaction with H3K9-methylated nucleosomes, whereas the CSD mainly functions as a dimerisation domain and a protein-protein interaction domain for HP1. The dimerisation surface can accommodate various proteins with a PxVxL motif. The interacting partners include transcriptional repressors (TRs) (e. g. transcription intermediary factor 1α (TIF1α), TIF1β/KAP-1, brahma-related gene 1 (BRG1)/SNF2β), a member of the Su(var)3-9 histone methyltransferase (HMTase) family, histone deacetylases (HDACs), a component of the basal transcriptional machinery ($TAF_{II}130$) and potentially transcriptional activators (TAs) (or trans-activating forms of TRs). Origin recognition complexes (ORCs) and some components of nuclear envelopes such as lamina-associated polypeptide 2β (LAP2β) and lamin B are known to interact with HP1 through the CD. The hinge region contains a number of phosphorylation sites and has RNA- and chromatin-binding activity. Also, the hinge region is involved in the interaction between HP1 and the inner centromere protein (INCENP).

Dynamic interaction of HP1 with chromatin

Because of reduced enzyme accessibility to heterochromatin [54] and the difficulty of extracting HP1 from the nucleus, it was speculated that the interaction between HP1 and chromatin is static and that HP1 could be acting as a 'molecular glue', locking nucleosomes tightly together and thereby blocking access of the transcription machinery or activators to genes that are buried within heterochromatic regions. This model has been challenged by several independent groups who have carried out photo-bleaching experiments on living cells with green fluorescent protein (GFP)-tagged HP1 variants [28, 55–57]. They showed relatively rapid exchange of

HP1 molecules in both heterochromatic and euchromatic regions. This implies that heterochromatin is not completely inaccessible to other molecules. Consistent with this, Verschure et al. have shown that compact chromatin domains are readily accessible to large macromolecules as analysed by testing the accessibility of condensed chromatin domains to proteins and dextrans of various molecular sizes in nuclei of living cells [58]. Hence, consistent with a mass action and probability model [59, 60], heterochromatin integrity and heterochromatin-induced gene silencing are likely to be maintained by dynamic competition between factors involved in the formation of an open chromatin structure and/or gene activation and those assisting the generation of a closed chromatin structure and/or gene repression. However, it must be noted that the mobility of HP1 is reduced and the fraction of more statically bound HP1 higher in heterochromatin than in euchromatin [55, 57]. This difference in mobility or amount of the 'immobile' fraction in these two regions may be due to differences in the density of HP1 binding sites. At heterochromatin where methyl H3K9 is abundant, an HP1 molecule is more likely to encounter its binding site. This may slow down the overall mobility of HP1. In support of this speculation, knocking out the *Suv39h1/2* genes in mice or mutations in Clr4 and Rik1 in *S. pombe* caused delocalisation and increased mobility of HP1 or Swi6 [28, 56]. Interestingly, it was shown, using *Drosophila* HP1 and a set of synthetic peptides containing mono-, di- or trimethylated H3K9 residues, that HP1 has different affinities for these differentially methylated forms of H3K9, in order of decreasing affinity tri > di > mono [61]. Although between species there are differences in nuclear distribution of differentially methylated H3K9, at least in mouse cells, the trimethylated form of H3K9 has been shown to be predominantly present at pericentromeric regions, whereas mono- or dimethyl forms localise predominantly at euchromatin or throughout nuclei (and some cases at the nuclear periphery), respectively [62–65]. Thus, the relative stability of HP1 binding to chromatin may also be influenced by the status of its binding site.

The interaction between HP1 and chromatin can be subjected to a drastic change when cells are physiologically stimulated and/or are undergoing cell division. We previously demonstrated that the immuno-activation of primary murine T cells in tissue culture can increase the mobility of HP1β as well as cause a reduction in the immobile or statically bound fraction in both hetero- and euchromatin [55]. In addition, the mobility and the amount of immobile fraction of Swi6 in yeast appears to change during different growth phases [56]. A number of studies investigating the behaviour of different HP1 isoforms during the cell cycle in mammals (mostly in interphase and metaphase) suggest that a significant relocalisation of HP1 occurs during the cell cycle. Despite the similarity in the primary amino acid sequences, HP1α, HP1β and HP1γ appear to have distinct localisation patterns in the interphase and mitotic nuclei. Although the predominant distribution of HP1α and HP1β is heterochromatic, whereas that of HP1γ is euchromatic, in interphase nuclei there is some overlap in the localisation of different HP1 isoforms [66, 67]. Recent studies in murine primary T lymphocytes have revealed that their activation causes HP1α (which normally has a relatively diffuse nuclear distribution compared to HP1β) to become more concentrated in and around the centromeric heterochromatin [68].

B cell activation was shown to result in upregulation of HMTases and their histone modifications as well as an increase in HP1β, the authors hypothesising that the relative lack of histone methylation in quiescent cells may be responsible for their increased propensity for reprogramming of transferred nuclei [69]. During metaphase of the cell cycle individual HP1 isoforms seem to localise and behave quite distinctly from one another. A number of studies confirmed that HP1α is the dominant HP1 species associated with the centromere of metaphase chromosomes [57, 66, 70, 71]. On the other hand, the centromeric localisation of HP1β and HP1γ is less certain. HP1β appears to associate with the centromere most prominently during anaphase rather than in metaphase [72]. In contrast, HP1γ seems to preferentially associate with distinct sites along the chromosomal arms in metaphase [66]. What causes this redistribution of HP1 variants on chromosomes? Phosphorylation of HP1 has been suggested as one possible triggering mechanism. It was previously shown in *Drosophila* that the HP1 primary amino acid sequence contains a number of consensus sites that can be phosphorylated [52]. All mammalian HP1 variants also contain a number of putative phosphorylation sites (mostly concentrated in the hinge) for a variety of kinases. Differentially phosphorylated species of *Drosophila* HP1a were identified during larval development [52, 73]. Similar observations were made for HP1α and γ but not HP1β in mammalian cell lines through the cell cycle [66]. Casein kinase II and Pim-1 kinase are candidate kinases for *Drosophila* HP1a and mammalian HP1γ phosphorylation, respectively [74, 75]. Mutational analysis of putative target sites on HP1 indicated that phosphorylation might regulate HP1 localisation and heterochromatin-mediated gene silencing [75, 76]. Apart from these kinases, TIF1α (transcriptional intermediary factor α) and TIF1β have been shown to phosphorylate all mammalian HP1 isoforms in vitro [39]. Furthermore, it has been proposed that the CD is required for HP1 localisation in interphase of the cell cycle, whereas during metaphase it is the CSD/hinge that is needed for correct HP1 localisation [70]. There is circumstantial evidence that post-transcriptional modifications of HP1 may affect the oligomerisation of HP1 with itself or various HP1-

Table 1. Examples of putative HP1-interaction proteins/molecules (modified from Li et al. (2002) [47])

Protein	Species	HP1 variant	Interacting domain	Reference(s)
Chromatin components/ modifiers and transcription regulators				
Histone H1	*Drosophila*	HP1	?	[34
Histone H3	*Drosophila*; mouse	HP1a, HP1α, HP1β, HP1γ	CD (for mouse HP1s)	[34, 49]
Methyl K9 histone H3	*S. pombe*; *Drosophila*;	Swi6, HP1a, HP1α, HP1β, HP1γ.	CD	[22, 25, 27]
mouse; human	$HP1^{Hs}\alpha$, $HP1^{Hs}\beta$, $HP1^{Hs}\gamma$	[23, 24]		
Histone H4	*Drosophila*; mouse	HP1a, HP1β	CSD (for HP1a)	[49, 154]
Members of	*Drosophila*; mouse;	HP1a, HP1α, HP1β, $HP1^{Hs}\beta$	CSD (for HP1a, HP1α)	[29, 108–110]
SUVAR3-9 HMTase	human			
Suvar3-7	*Drosophila*	HP1a	CSD	[155, 156]
Suvar4-20 HMTase	mouse	HP1α, HP1β, HP1γ	?	[157]
Su(z)12	human	$HP1^{Hs}\alpha$, $HP1^{Hs}\gamma$	CSD	[43]
Dnmt3a	mouse	HP1α	?	[102, 103]
Dnmt3b	mouse, human	HP1α, HP1β, $HP1^{Hs}\alpha$, $HP1^{Hs}\beta$	?	[62, 102]
Kap-1/TIF1β	mouse, human	HP1α, HP1β, HP1γ; $HP1^{Hs}\alpha$, $HP1^{Hs}\gamma$	CSD	[20, 39–42]
TIF1α	mouse	HP1α	CSD	[39, 41]
SNF2β/BRG1	mouse	HP1α	CSD	[100]
ATRX	mouse	HP1α, HP1β	CSD	[101]
$TAF_{II}130$	human	$HP1^{Hs}\alpha$, $HP1^{Hs}\gamma$	CSD	[44]
RNA	mouse	HP1α, HP1γ	Hinge (for HP1α)	[36]
Chromatin	*Xenopus*	xHP1α	Hinge	[51]
Proteins involved in DNA replication/chromosomal segregation/cell cycle				
CAF-1 (p150 subunit)	mouse, human	HP1α, HP1β, $HP1^{Hs}\alpha$	CSD	[20, 42, 45]
Ku70	human	$HP1^{Hs}\alpha$	CSD	[158]
ORC1-6	*Drosophila*	HP1a	CD, CSD	[48]
HOAP	*Drosophila*	HP1a	?	[91, 92]
Psc3	*S. pombe*	Swi6	CD	[81]
INCENP	human	$HP1^{Hs}\alpha$, $HP1^{Hs}\gamma$	Hinge (for $HP1^{Hs}\alpha$)	[50]
Hsk1/CDC7	*S. pombe*	Swi6	?	[83]
Proteins involved in nuclear organisation				
Nuclear envelope	mouse	HP1α, HP1β, HP1γ	CD	[88]
Lamin B receptor	human	$HP1^{Hs}\alpha$, $HP1^{Hs}\beta$, $HP1^{Hs}\gamma$	CSD	[49, 89]
Lamin B	mouse	HP1β	CD	[88]
LAP2β	mouse	HP1β	CD	[88]

interacting proteins, thereby altering its localisation or the function of its CD and CSD [39, 52, 73]. However, the mechanisms involved in such re-organisation of HP1 or changes in the stability of HP1-chromatin interaction remain to be elucidated.

HP1 function and HP1-interacting proteins

Identification of various HP1-interacting proteins enabled us to speculate how HP1/heterochromatin might be involved in centromere function, nuclear organisation, chromatin assembly and gene regulation. Examples of HP1-interacting partners are listed in table 1.

Centromere and telomere function

Centromeres of higher eukaryotes and the fission yeast, *S. pombe*, consist of repetitive DNA sequences and are associated with HP1. The *S. pombe* homologue of HP1, Swi6, is required for efficient cohesion between sister chromatids during the cell cycle [77]. Similarly,

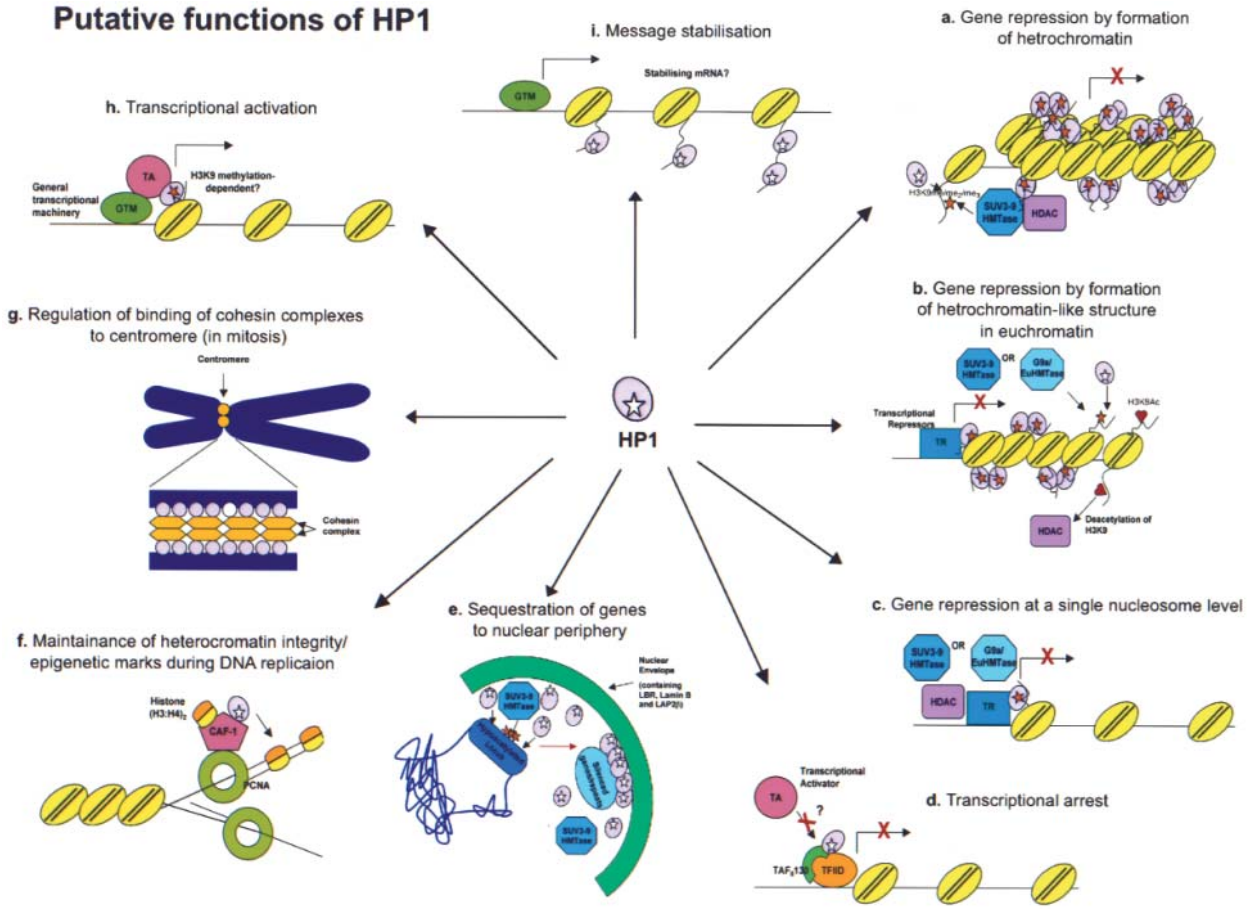

Figure 2. A summary of the suggested functions of HP1. (A) At heterochromatic regions, HP1 dimers cross-link a regularly positioned array of H3K9 methylated nucleosomes. The interaction of HP1 with Su(var)3-9 histone methyltransferase(HMTase) and/or histone deacetylases (HDACs) creates the positive feedback loop for formation and maintenance of a highly compact chromatin structure. A gene that is buried in such a condensed chromatin environment is likely to be unavailable for active transcription. (B) In euchromatic regions, the HP1/transcriptional repressor (TR) complex mediates gene repression by binding to a specific site of a locus and propagating the formation of a higher-order chromatin structure along the locus. This may be accompanied by the action of Su(var)3-9 or other euchromatic H3K9 HMTases (e.g. G9a) and/or HDACs. (C) The gene repression by HP1 or an HP1/TR complex may be accomplished in a short-range/at a single nucleosome level as seen with cyclin E locus. (D) The interaction of HP1 with TATA-binding protein (TBP)-associated factor of 130 kD ($TAF_{II}130$) in the context of TFIID may prevent the association of the general transcriptional machinery (GTM) or factors with the transcriptional activator, resulting in transcriptional arrest/gene repression. (E) The genes or repetitive DNA elements that have been silenced by heterochromatin formation, possibly involving the HP1-H3K9 methylation mechanism, can be sequestered to the nuclear periphery by the interaction of HP1 with components of the nuclear envelope [e. g. lamin B, lamin B receptor (LBR), lamina-associated polypeptide 2β (LAP2β)]. The localisation of a gene to the heterochromatin at the nuclear periphery may result in increased availability of the factors necessary for heterochromatin formation/gene silencing. A gene that is undergoing the process of silencing (e. g. a hypoacetylated locus) may be recruited towards the nuclear periphery to complete that process and be sequestered at that site. (F) HP1 interacts with the large subunit of chromatin assembly factor 1(CAF1), p150. CAF1 transports a tetramer of histone (H3/H4) to the replication fork via interaction with proliferating cell nuclear antigen (PCNA). The recruitment of HP1 to the replication fork in late S-phase of the cell cycle may be important to maintain both heterochromatin integrity (especially at centromeres and telomeres) and possibly the epigenetic status of silenced genes. (G) During mitosis, the interaction between chromatin-bound HP1 and a component of the cohesin complex, Psc3 (in *S. pombe*) is required for the appropriate localisation and/or loading of sufficient cohesin complexes to the centromeres of sister chromatids on chromosomal segregation. (H) HP1 may be involved not only in transcriptional repression/gene silencing but also in transcriptional activation. The interaction of HP1 with a transcriptional activator or trans-activating form of transcription intermediary factor 1α/β (TIF1α/β) or brahma-related gene 1 (BRG-1/SNF2β), and recruitment of such a complex to a specific site of a gene may induce active transcription. The recruitment of the HP1/transcriptional activator (TA) complex can be H3K9 methylation-dependent or independent. (I) HP1 may work as a 'message stabiliser' by binding nascent mRNA at the site of transcription.

in *Drosophila,* HP1 is required for faithful chromosome segregation [78]. It has been shown that mutating Swi6 increases the incidence of lagging chromosomes and loss of chromosomes during mitosis [79]. Furthermore, the loss/reduction of histone H3K9 methylation led to increased chromosomal instability due to a high frequency of non-homologous pairing in male meiosis in Suv39h-null mice [80]. The link between HP1 and

centromere function has been explained by a study in *S. pombe* that revealed a physical interaction between Swi6 and the cohesin protein complex [81]. The mitotic cohesin complex of *S. pombe* is composed of three protein subunits, Rad21, Psc3 and Pms1, which are enriched at the outer centromeric DNA repeats, bringing about adhesion of the centromeres of sister chromatids. In a pull-down assay, it was shown that Psc3 physically interacts with Swi6 and mouse HP1α. Moreover, using chromatin immunoprecipitation, significantly reduced binding of Psc3 at the outer centromeric repeats and mating-type loci was shown in mutant strains for *swi6* and *rik1* (responsible for H3K9 methylation together with Clr4 [82]). In addition, it was recently shown that a DNA replication regulator/initiator, Hsk1(CDC7)-Dfp1 interacts with and phosphorylates Swi6 [83]. Phosphorylation of Swi6 by Hsk1-Dfp1 appears to be necessary for association of Swi6 with other molecules, including Psc3, for appropriate cohesion at centromeric heterochromatin [83]. HP1 thus appears to assist localisation and/or loading of sufficient cohesin molecules onto sister chromosomes for homologous pairing and proper chromosomal segregation (fig. 2G). Interestingly, HP1 also binds to telomeres and is required to prevent telomere-telomere fusions. Recent *Drosophila* studies suggest that neither H3 K9 methylation nor the HP1 chromodomain are necessary for telomere capping, but that instead HP1 appears to bind directly to the telomeric DNA [84]. In contrast, it was shown that telomere silencing was dependent on the recruitment of Suv39 and the HP1 chromodomain. However, it is not clear whether the role of HP1 proteins in telomeric DNA capping is conserved in other eukaryotic organisms. This is because *Drosophila* prevents telomere attrition via DNA transposition. Therefore, *Drosophila* telomeres lack the DNA repeats that are inserted by telomerase in other eukaryotes.

Nuclear organisation

In several types of murine and human cells (especially in hematopoietic cells), centromeric heterochromatin is frequently found localising at the nuclear periphery [85, 86]. In addition, it has been shown that developmentally silenced/repressed genes are recruited to the nuclear periphery [87]. This localisation pattern may be due to an interaction between HP1 and components of the nuclear envelope [88]. It was shown by immunofluorescent staining that HP1 co-localises with the nuclear envelope. Furthermore, the interaction of HP1 with constituents of the nuclear envelope such as the LBR [49, 89], lamin B and LAP2β [88] was demonstrated using a binding assay and yeast two-hybrid assays. In the case of LBR, the interaction could be direct or indirect and seems to involve the core histones: H3 and H4 [49]. Interestingly, treatment with TSA or in vitro hyperacetylation of the core histones by CREB-binding protein negatively affected the interaction between LBR and HP1. The proposed model suggests that the LBR:HP1:H3/4 complex can exist either as a fully assembled complex or as a partially dissociated complex. Therefore, an already-hypoacetylated gene that is destined to be silenced could be recruited to the nuclear periphery where HP1 and other heterochromatin-forming factors might complete the silencing process. The sequestration of the silenced gene to the nuclear periphery may be dependent on HP1-binding (fig. 2E). Thus, HP1 might tether heterochromatin and possibly some silenced genes to the nuclear periphery, thereby allowing transcriptionally active regions to move more efficiently into transcription factories in the inner part of the nucleus [90].

Chromatin assembly

In addition to cohesin and the nuclear envelope, HP1 is known to associate with proteins involved in chromatin assembly during DNA replication and repair. Pull-down and immuno-precipitation assays showed that the p150 component of chromatin assembly factor 1 (CAF1) interacts with murine HP1α and HP1β or human $HP1^{Hs}\alpha$ [20, 42, 45]. As mentioned earlier, this CAF1 subunit contains the consensus PxVxL motif, which interacts with the CSD in vitro. However, the physiological importance of the CAF1-HP1 interaction is still unclear because mutations in the HP1-binding motif prevent the interaction between CAF1 p150 and HP1 but do not affect the recruitment of CAF1 p150 to replication foci in either heterochromatin or euchromatin through S-phase [45]. Nevertheless, a recent study by Quivy et al. revealed the existence of a replication-specific pool of HP1α that associates with CAF1 proteins through its large subunit, p150. Interestingly, this HP1α/CAF1 complex does not contain histone H3/H4. This pool of HP1α is localised, in an H3K9 methylation-independent manner, to the periphery of pericentromeric heterochromatin domains, which are the sites of ongoing DNA replication. This localisation of HP1α appears to be dependent on CAF1 p150, since knock-down of p150 with small interfering RNA (siRNA) abolishes the HP1α staining from these sites of ongoing replication. These findings suggest that CAF1 may somehow promote the incorporation of HP1 into pericentric heterochromatin during its replication in late S-phase (fig. 2F). *Drosophila* ORC and HP1/HOAP have also been found to associate with HP1 in co-immuno-precipitation assays [48, 91, 92]. These proteins are thought to be necessary for heterochromatin assembly since mutations in their genes suppress PEV [48, 91, 92]. In *Saccharomyces cerevesiae* (with lacks HP1), ORC interacts with silent information regulator (SIR) proteins to propagate formation of silent domains at silent mating-type loci [93–97]. By analogy, mutations

in ORC cause disruption of heterochromatic localisation of HP1 in *Drosophila* [73]. HOAP resembles DNA sequence-specific high mobility group (HMG) proteins at the primary amino acid sequence level, and, at least in vitro, it binds to AT-rich satellite repeat sequences [91]. Interestingly, an innovative approach to localise chromosomal proteins by expressing them as fusion proteins with a DNA adenine methyltransferase, known as DAM-ID, revealed that HP1a and HP1c are enriched at AT-rich DNA sequences in *Drosophila* Kc cells [98]. Treatment with a peptide that interferes with HOAP-HP1 interaction displaced HP1 from the entire *Drosophila* polytene chromosome [92]. Like SIR proteins, HP1 does not recognise a specific DNA sequence, so that ORC/HOAP-HP1 association may be required for developmentally regulated targeting of HP1 to specific sites in the genome to nucleate heterochromatin assembly. Moreover, recent studies demonstrate that specific repetitive elements on the fourth chromosome in *Drosophila* seem capable of nucleating heterochromatin formation/silencing that is sensitive to HP1 dosage [99].

HP1 as a regulator of gene expression

Several HP1-interacting proteins may reflect HP1's mode of action in gene regulation. An example of one such protein is Su(var)3-9 and its mammalian homologues. Moreover, transcriptional co-repressors/chromatin remodelling complex Su(z)12 [43], TIF1α, TIF1β/KAP-1 [20, 39–42], brama-related gene 1 (BRG1)/SNF2β [100], ATRX [101]), subunits of the basal transcriptional machinery ($TIF_{II}130$) [44] and DNA methyltransferases (Dnmt3a/b [62, 102, 103]) can physically associate with one or more HP1 variants either directly or in protein complexes. We shall discuss the relation between some of these proteins and HP1 function in detail in the next section.

HP1 at heterochromatin

The large-scale mutagenesis analysis of PEV not only led to the identification of HP1 as a structural constituent of heterochromatin but also as a molecule which plays a crucial role in gene silencing in PEV. Mutations in the *Su(var)2-5* gene, which encodes *Drosophila* HP1, suppressed the variegation of the *white* gene when translocated from its normal euchromatic location close to centromeric heterochromatin [9, 17].

A detailed study revealed that the silenced gene had acquired more heterochromatin-like features than its euchromatic counterpart [104, 105]. The features included more resistance to nuclease digestion and organisation of the locus into a regular and dense nucleosome array. The nuclease resistance of a variegating gene was also observed in transgenic mice carrying the human CD2 reporter gene with a disabled locus control region (LCR; a dominant *cis*-acting element that exerts position-independent, copy number-dependent expression of transgenes in mice) [106]. HP1 appears responsible for formation of such tight chromatin structures, since in *Drosophila* a variegating gene was more accessible to restriction enzymes on an HP1 mutant background [107]. It has been shown in PEV models of *Drosophila* and mammals that overexpression of HP1 often results in enhancement of variegation [8]. These observations suggest that HP1 can influence gene silencing, at least at a certain time in development, when the decision is made for a variegating gene to be expressed or repressed. HP1 can potentially propagate the formation of a heterochromatic structure, since it has been demonstrated that HP1 and SUV3-9 homologues can physically interact, at least in *Drosophila* and in an in vitro assay with mouse HP1α/β and human/mouse SUV39h1 [29, 108–110]. Thus, more HP1 at a particular locus would lead to recruitment of more SUV3-9 and vice versa so that the presence of HP1 or SUV3-9 in a particular nuclear environment might spread the formation of a heterochromatin structure in the absence of dominant *trans*- or *cis*-activating factors/elements [111, 112]. Moreover, HP1 and SUV3-9 homologues may recruit DNA methyltransferases (Dnmt3a/b and with SUV3-9, also Dnmt1) and histone deacetylases (HDACs) [113] by physical interaction, resulting in the generation of other epigenetic (heritable influence on gene activity, unaccompanied by a change in the DNA sequence itself) marks involved in stable gene silencing. So far, there is no evidence for the direct interaction between HP1 and HDACs, whose action would facilitate the methylation of histone H3K9 by deacetylating pre-acetylated K9 residues. However, it is known that HDAC activity is indirectly recruited to an HP1-rich domain via SUV3-9 [113, 114] or a component of a TIFβ/KAP-1-containing transcriptional repressor complex [115]. Taken together, the repressive effect of pericentric or telomeric heterochromatin on gene activity is likely to be mediated by the generation of densely methylated H3K9 marks and HP1 binding which results in tight cross-linking of nucleosomes, thereby facilitating formation of a highly condensed chromatin structure [22] (fig. 2A). This is probably an efficient way of silencing a gene located in a heterochromatic environment where all the factors needed are abundant.

HP1 in euchromatin

In *Drosophila* and mammals, it has been demonstrated that HP1s also localise and can exert a repressive effect in euchromatic environments [12, 98, 116–118]. But does HP1 use the same trick to silence genes in euchromatic regions where the local concentration of heterochro-

matin-forming factors could be low? In HP1 tethering experiments, using a Gal4-HP1 chimeric protein and the *mini-white* transgene containing Gal-4 binding sites upstream of the promoter, the effect of tethering HP1 has been tested at various euchromatic sites in *Drosophila* [119]. Only one out of six genomic sites resulted in variegation of the *mini-white* gene. This sole site happened to be in the middle of repetitive DNA elements that are also present in heterochromatic domains. In another experiment, using a lac operator-repressor system in the place of Gal-4, tethering resulted in repression of the *white* reporter gene at 25 out of 26 genomic sites [120]. The site unaffected by HP1 tethering happened to be located upstream of an active gene. Although these two experiments show opposite efficiency of reporter repression at euchromatic sites, both indicate that chromosomal context may be important for HP1 function in euchromatic environments. Tethering studies were also carried out with human and murine HP1 using a smaller number of tethering sites (4–6, compared with experiments in *Drosophila* in which the number varied from 3 to 256) [44, 121]. Tethering of mammalian HP1 species led to transcriptional repression of reporter genes. In addition, it was recently shown in a CHO-derived cell line that tethering of EGFP-tagged HP1α/β-lac repressor fusion proteins to a lac operator-containing chromosomal region led to the localised formation of a condensed chromatin structure. Such chromosomal condensation was accompanied by recruitment of SETDB1 (another H3K9-specific HMTase) and an increased level of H3K9 trimethylation [122] (fig. 2B). However, more striking is the finding that the repressive effect of tethering seems to be short-range, since locating the tethering sites more than 2 kb away from the promoter of the reporter gene abolishes the effect [121]. This suggests that HP1 can exert silencing on a gene-by-gene basis. In fact, a few endogenous genes have been shown to be silenced by HP1 on this basis, and the gene-specific recruitment of HP1 involves its association with transcriptional regulators that bind specific DNA sequences [12–14]. For example, it has been demonstrated that the promoter of E2F- and myc-responsive genes such as E2F-1, c-myc and cdc25A are associated with $HP1^{Hs}\gamma$ and repressed in quiescent cells [12]. The targeting of $HP1^{Hs}\gamma$ to these promoters is mediated by interaction with the E2F-6 complex containing G9a and EuHMTase, both of which have HMTase activity specific for histone H3K9. Furthermore, HP1 was also shown to be specifically targeted to the cyclin E promoter by Rb protein [13]. Apart from HP1, Rb also interacts with SUV39: the repression of the cyclin E gene seems to be dependent on the activity of this enzyme [13]. In this case, HP1 binding was completely eliminated when targeted only 0.5 kb upstream of the promoter. This indicates that HP1 may be mediating formation of a small heterochromatin-like structure at specific sites on a locus (fig. 2C). Studies on the endogenous *col11a2* gene, whose expression is repressed by an HP1/NT2 KRAB zinc-finger protein complex, support of this idea. The binding of HP1α/HP1γ and SETDB1 and the methyl H3K9 mark were found to 'spread' over a short distance from the NT2 binding site that acts as the 'nucleation centre' [14]. Alternatively, HP1 might be able to mediate repression by mechanisms unrelated to formation of heterochromatic structures. Interestingly, it has been shown that methyl H3K9 alone is not sufficient to recruit HP1 to a reporter gene, although methyl H3K9 can repress the reporter gene independently of HP1 through histone deacetylation [123]. Consistent with the above examples of HP1/methyl H3K9-mediated gene repression, this suggests that recruitment of HP1 to a specific gene may require the interaction of HP1 with a complex containing a DNA sequence specific-binding protein and an H3K9 HMTase. Recently it was shown that the interaction of TIF1β/KAP1 with HP1 was required for progression through differentiation in F9 cells by regulating the expression of key endoderm differentiation genes [124]. It has been demonstrated in mouse embryonic fibroblasts (MEFs) that Rb-dependent repression of cyclin E is mediated through histone deacetylation of a single nucleosome located at the transcriptional start position [125] (fig. 2C). Moreover, looking at nucleosome positioning in the 5' region, nucleosomes are organised rather sparsely with no difference between wild-type and Rb knockout MEFs, indicating that Rb-mediated HP1 binding to the cyclin E promoter may not induce repression by heterochromatin-like structure formation but by a more refined, unknown mechanism. Similar speculation could be applied to the situation where genes are repressed by association of $TAF_{II}130$ with HP1. $TAF_{II}130$ is a component of the general transcription factor, TFIID complex, and interacts with HP1α and HP1γ in the context of TFIID in a mammalian cell line [44]. It is possible that HP1/$TAF_{II}130$ prevents association of TFIID with transcriptional activators (fig. 2D). Or a DNA-bound HP1/TFIID complex could be sustained in a repressed status to prohibit rapid activation.

HP1 in RNAi-mediated gene silencing

Studies in *S. pombe* have shown that RNA interference (RNAi) is required for the establishment and maintenance of silenced heterochromatin domains [126, 127]. Deletions of components of RNAi machinery, including Argonaute (Ago), Dicer (Dcr) and RNA-directed RNA polymerase (Rdp), caused derepression of centromeric repeats [126] and some meiotic genes that are normally silenced during vegetative growth [127]. This derepression is accompanied by loss of H3K9 methylation and Swi6/HP1 binding. Similarly, in *Drosophila*, mutations in *aubergine*, *piwi* and *spindle-E/homeless* genes that

encode RNAi components resulted in derepression of the variegating white transgenes, abnormal localisation of HP1 and reduction in H3K9 methylation on polytene chromosome [128]. Consistent with these observations, derepression of centromeric repeats, together with reduced H3K9 di- and tri-methylation and de-localisation of HP1β/HP1γ, has been described in Dicer-null mouse ES cells [129]. To examine the involvement of Swi6/HP1 in RNAi in *S. pombe*, a short hairpin RNA which targets *Ura4* and results in silencing of the *Ura4* gene in the wild-type strain was expressed in a *Swi6* deleted strain. This experiment showed that the production of siRNA was not affected in the absence of Swi6 but that the spreading of the heterochromatic domain (defined by H3K9 methylation) from the target site along the *Ura4* locus was greatly inhibited [127]. Taken together, HP1 does not appear to be involved in the initiation of RNAi but seems to work downstream of the RNAi pathway and may be necessary to propagate the silencing effect along a particular chromsomal region in concert with the action of Suv3-9 HMTase.

HP1 as a transcriptional co-activator?

Recent observations suggest that association of HP1 and/or Suv3-9 HMTase with chromatin often correlates with heterochromatin-like structures and gene silencing. However, if one takes into account the chromosomal context and nuclear environment of a particular locus, this correlation may not be so simple. Examples of HP1 apparently acting as a transcriptional activator are described in *Drosophila Su(var)2-5* mutant larvae. Expression of the essential heterochromatic genes, *rolled* (*rl*) and *light* (*lt*) is reduced at the messenger RNA (mRNA) level when HP1 is mutated [11]. Moreover, the relocation of the *lt* gene from its heterochromatic location to a euchromatic region results in variegation of *lt* [130]. In addition, the DAM-ID microarray analysis by Griel et al. confirmed that naturally pericentric genes that are expressed at medium to high level are bound by HP1 and Su(var)3-9 [98]. A detailed analysis in *Drosophila* showed that several genes that are downregulated by reduction in HP1 level bind to HP1 in normal circumstances [131, 132]. In some cases, the interaction seems to be independent of the action of Suv3-9. This may suggest that HP1 can act as a transcriptional activator in its own right [131]. Furthermore, overexpression of HP1β resulted in dose-dependent suppression of variegation in hCD2 transgenic mice that exhibit variegation despite having their transgenes integrated in the long arm of the chromosome [8]. With these phenotypic assays, however, it is hard to tell whether the effect is exerted directly or indirectly by HP1. Assuming the effect is direct, one explanation would be interaction of HP1 with both known- and still unknown transcriptional activators (fig. 2H). HP1-associating transcriptional regulators such as TIF1s and BRG1/SNF2β can act as both transcriptional co-repressors and co-activators [39,133,134]. If these proteins bind to HP1 in a co-activator form, it is possible for HP1 to act also as a co-activator. In the case of TIF1s, they may phosphorylate HP1 on binding, thereby altering its function [39,135], though there is no evidence for any functional importance of HP1 phosphorylation by TIFs. There are also several possible explanations for how overexpression of HP1 suppresses variegation in a euchromatic environment. First, overexpression of one type of HP1 variant may shift the abundance of various HP1 homo-/heterodimers in vivo. It must be noted that some HP1-interacting co-repressors interact preferentially with a specific HP1 variant(s) [100] and in some cases, the interaction requires CSD dimerization [46]. Overexpression of a single HP1 variant may interfere with the formation of repressor-interactive dimers. The second possibility is that overexpressed HP1 titrates repressor molecules in euchromatic regions and recruits and/or sequesters them to heterochromatin regions. In addition to these speculations, a novel mechanism for HP1-mediated gene regulation was recently suggested [15]. They have demonstrated association of HP1 with transcriptionally active and heat-shock-induced puffs on the *Drosophila* polytene chromosome. Intriguingly, they found that HP1 was not binding at promoter regions but at the coding regions, and that this association required the presence of RNA transcripts since treatment with RNase or sodium salicylate (which induces heat-shock puff formation without RNA transcription) diminished the recruitment of HP1 to heat-shock-induced puffs. Moreover, the levels of the affected Hsp70 transcript or protein correlated with HP1 dosage after 3 h of heat shock, but after 7 h the correlation becomes inverted, suggesting that HP1 assists active transcription and efficient recovery from the transcription. It is unclear whether HP1 regulates mRNA levels by altering the stability of mRNA or by controlling the rate of transcription (fig. 2I) but in any case, these findings suggest a new role for HP1 in gene regulation.

HP1-methyl H3K9 mechanism in gene silencing: heritability vs plasticity

As discussed above, the HP1-methyl H3K9 complex seems to be involved in both heritable gene silencing (conventional PEV) and repression of inducible genes. In the case of the latter, these genes are transcriptionally activated in response to physiological stimuli, including growth factors, cytokines/chemokines, stress and surface-receptor cross-linking. Methylation marks on histone tails are known to be much more stable than other modifications, such as acetylation/deacetylation. Moreover, although a histone H3K4-specific demethylase was recently identified [136], it is not clear whether

demethylases exist that are specific for other lysine residues. If this is the case, how do such genes switch their transcription status? According to a hypothesis suggested by Fischle et al., H3K9 may function in the context of a 'modification cassette' together with the adjacent serine 10 (H3S10) residue [137]. It has been reported that phosphorylation of H3S10 involves the extracellular signal-related kinase/mitogen-activated protein kinase (ERK/MAPK) and p38 signalling cascade [138–140] and cytokine-induced NFκB pathway [141, 142]. Several kinases have been identified as putative histone H3 kinases, including mitogen and/or stress activated kinases such as Msk1 and RSK2 that are involved in the H3S10 phosphorylation and activation of immediate-early genes [138, 140]. Moreover, an inhibitor of NFκB kinase α (IKKα) that works downstream of the NFκB pathway has been shown to phosphorylate H3S10 in vitro and is also necessary for a gene-specific H3S10 phosphorylation on NFκB-dependent genes in vivo [141, 142].

Structural analysis of the HP1 CD and an in vitro assay suggest that phosphorylation of H3S10 significantly reduces the binding affinity of HP1 to methyl H3K9 [137]. Consistent with these observations, immuno-activation of murine primary T-cells does not only increase the mobility and reduce the immobile fraction of HP1β but also derepresses a T-cell-specific reporter transgene which is silenced by PEV [55; K. Hiragami and A. Saveliev, unpublished observation]. Thus, phosphorylation of H3S10 and HP1 displacement may be essential for the activation of some genes. This hypothesis has recently been tested by Mateescu et al. Unexpectedly, their findings suggest that the dual modification of H3K9 methylation and H3S10 phosphorylation does not reduce the affinity of HP1 to chromatin but instead increases the interaction [143].

The dissociation of HP1 from chromatin appears to require an additional modification, H3K14 acetylation. This is not entirely surprising, as previous studies have shown that activation of a number of immediate-early genes is accompanied by phosphoacetylation of histone H3**.** Taken together, both phosphorylation and acetylation of histone H3 may be necessary to overcome HP1- and/or methyl H3K9-mediated gene repression. Moreover, in most cases, inducible genes need to be repressed promptly when cells are no longer receiving activation signals. In this model, the activation of genes can occur without altering methyl H3K9 status. In this way, the induced gene might be shut down relatively easily by dephosphorylation of H3S10. For this dephosphorylation, a type 1 serine/threonine protein phosphatase (PP1) may be a good candidate. The catalytic subunit of PP1 is encoded by *Su(var) 3-6,* whose mutation led to derepression of the *mini-white* transgene and abnormal mitosis (with overcondensed metaphase and anaphase chromosomes) in *Drosophila* [144, 145], indicating that the targets of PP1 may include phospho-H3S10.

However, one might wonder how gene activation can occur in the presence of methyl H3K9? It has been reported that histone modifications associated with active transcription, including H3K4 methylation [146, 147], are mutually exclusive to H3K9 methylation [146]. There are, however, some intriguing in vitro data showing that histone modifications with opposing functions may exist on the same histone H3 tail. Apart from SUV3-9 homologues, H3K9 methylation can be accomplished by G9a, SETDB1 and EuHMTase [148–150]. Interestingly, unlike SUV39H1, G9a and SETDB1 can methylate H3K9 on a histone tail that has been already methylated at K4 in vitro [150, 151]. Histone H3K4 methylation is normally associated with active transcription and known to repel transcriptional repressor complexes at the site of modification and to enhance histone acetylation [151–153]. K4 methylation is achieved by SET7/9 [151, 152]. This enzyme can also methylate a pre-K9-methylated H3 peptide in vitro [151]. If these in vitro observations apply in vivo, the same H3 tail can be dually methylated at both K4 and K9. The presence of HP1 on H3K4/K9 methylated nucleosomes may prohibit the effect of methyl H3K4 and/or the binding of HP1 with methyl H3K9 may interfere with the action of SET7/9. The displacement of HP1 by H3S10 phosphorylation and/or H3K14 acetylation may release chromatin or nucleosomes from these prohibitions, resulting in gene activation.

Conclusion

It is more than a decade since HP1 was first discovered and found to influence gene silencing. Key links between histone modifications and the localisation of HP1 to specific chromosomal regions have provided the basis for molecular models of its in vivo function. Moreover, HP1 seems to provide connections by interacting with proteins involved in a variety of nuclear processes. We now know more about the role of HP1 in a heterochromatic environment, but how HP1 exerts its effects on gene expression/repression in euchromatic environments is largely unknown. The focus is now shifting to elucidate these mechanisms. It is certain that HP1 will remain popular in the field of epigenetics for some time to come.

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