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HP1: a functionally multifaceted protein

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HP1 (heterochromatin protein 1) is a nonhistone chromosomal protein first discovered in *Drosophila melanogaster* because of its association with heterochromatin. Numerous studies have shown that such a protein plays a role in heterochromatin formation and gene silencing in many organisms, including fungi and animals. Cytogenetic and molecular studies, performed in *Drosophila* and other organisms, have revealed that HP1 associates with heterochromatin, telomeres and multiple euchromatic sites. There is increasing evidence that the different locations of HP1 are related to multiple different functions. In fact, recent work has shown that HP1 has a role not only in heterochromatin formation and gene silencing, but also in telomere stability and in positive regulation of gene expression.

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Introduction

HP1 (heterochromatin protein 1) is one of the most intensely studied proteins in the chromatin field. Some might ask why so many people are working so hard on this protein instead of other chromosomal proteins. The answer is that this protein holds people's interest because it continuously discloses new and surprising features.

HP1 is a chromosomal protein first discovered in *Drosophila melanogaster* because of its association with heterochromatin; subsequent analysis showed that mutations in the gene for HP1 suppressed the silencing effect of heterochromatin in position effect variegation (PEV) [1••,2,3]. Molecular studies have shown that HP1 is phylogenetically highly conserved, present in many eukaryotes, including fission yeast, insects and mammals, consistently associated with heterochromatin and telomeres and involved in gene silencing [4,5].

In *Drosophila*, HP1 is encoded by the *Su(var)2-5* gene, which acts as a dosage-dependent modifier of position effect variegation [3]. This gene is composed of five exons separated by four introns (Figure 1). HP1 is a 206 amino acid protein with two prominent structural motifs, the chromo domain [6] and the chromoshadow domain [7], which are thought to be important for chromatin binding and protein interactions, respectively. The two domains are connected by a linker called the hinge (Figure 1).

A detailed cytological analysis of polytene chromosomes of larval salivary glands (Figure 2a) has shown that in *Drosophila*, HP1 is located not only at the pericentric heterochromatin but also on about 200 mapped regions along the euchromatic arms, and is a stable component of all of the telomeres [8•]. As summarized in Figure 2b, several recent studies collectively have shown that these three different positions are related to three different functions of HP1: heterochromatin formation and gene silencing, telomere capping and silencing and positive control of gene expression.

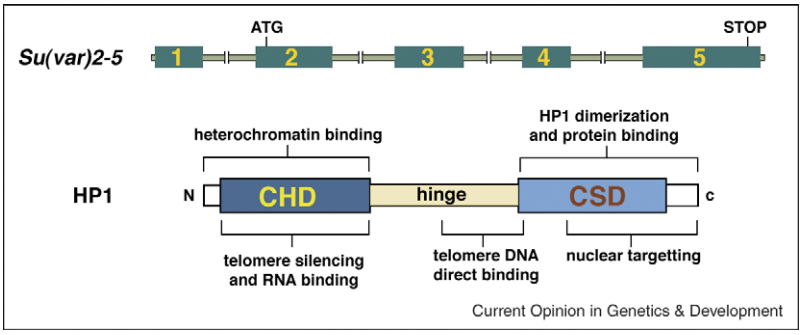
In the present review, owing to space limitations, while other HP1 homologues (HP1b and HP1c) have been discovered in *Drosophila* (and in mammals), we will mainly discuss the functional aspects of HP1 (HP1a) in *Drosophila*. For a more complete view of HP1's role in diverse aspects of genome metabolism, and the evolution of the HP1 family, we suggest some recent very good reviews that describe and discuss the mass of data obtained in various organisms [5,9,10•].

Role of HP1 in heterochromatin formation and gene silencing

Heterochromatin is a nearly ubiquitous component of the eukaryotic chromosome that is usually located at the pericentromeric regions and telomeres [11,12]. Studies on position effect variegation have suggested that heterochromatin can spread and act over great distances, inducing an epigenetic gene repression that can persist on the chromosomes through multiple mitoses. The 'heterochromatization' model for the epigenetic gene silencing observed has been supported by the isolation and identification in *Drosophila* of several modifiers of PEV [dominant suppressors of variegation (*Su(var)*) and dominant enhancers of variegation (*E(var)*)], in addition to the HP1-encoding *Su(var)2-5* gene, which correspond to trans-acting components affecting chromatin structure and/or function [13].

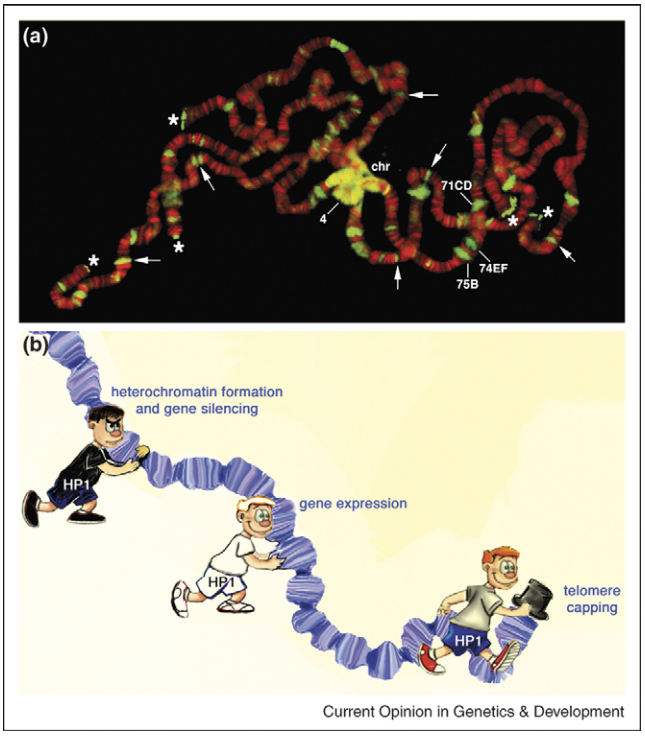
Although different sets of data have shown that HP1 may interact with many different proteins [5], until recently we lacked precise molecular models to explain how HP1

Figure 1



Diagrammatic representation of the structure of the *Drosophila Su(var)2-5* gene and its product HP1. The gene is made up of five exons separated by four introns. The positions of the ATG and STOP codons are indicated. The protein is made up of the chromodomain (CHD) at the N terminus (N) and the chromoshadow domain (CSD) at the C terminus (C) linked by the hinge region. The functional roles of the different domains are also indicated. The chromodomain binds the H3 N-terminal tail if methylated at lysine 9; the chromoshadow domain dimerizes and binds the SU(VAR)3-9 HMT or other proteins.

Figure 2



The chromosomal distribution of HP1 in *D. melanogaster* is related to its different functions. **(a)** The immunostaining of larval polytene chromosomes with a specific antibody against HP1 reveals that this protein is associated with the heterochromatic chromocenter (chr), the small fourth chromosome (4), all of the telomeres (asterisks) and numerous euchromatic sites (see arrows for examples) including the physiological puffs (75B, 74F, 71CD). **(b)** The cartoon diagrammatically represents the different functions of HP1 in the different chromosomal locations.

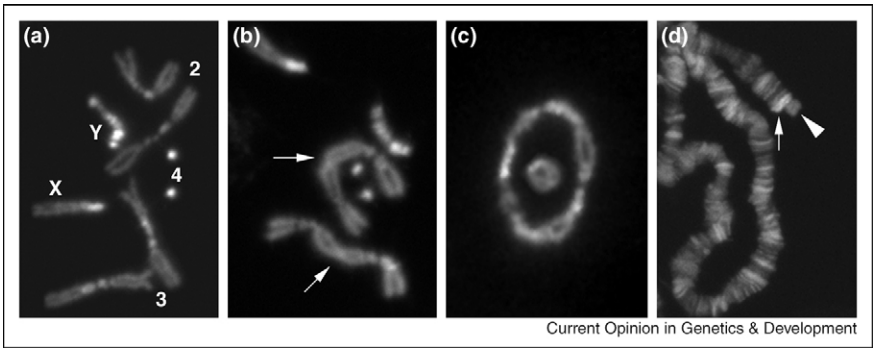
domains could recognize chromatin, mediate protein–protein interactions and induce heterochromatization and gene silencing. The specific HP1-interacting histone methyltransferase (HMTase) SUV39H1/Clr4 [14–17], first identified in mammals and yeast, respectively, are homologues of the *Drosophila* PEV modifier HMTase suppressors of variegation SU(VAR)3-9 [18]. This has suggested a model in which the interactions among an HMTase, modified histone H3 and HP1 are the underlying basis for heterochromatin formation and epigenetic gene silencing. According to the model, the SU(VAR)3-9 enzyme methylates histone H3 at Lys 9 (K9), creating a selective binding site for the chromo domain of HP1 while interacting with HP1 through its chromoshadow domain. This three-component complex then forms a specialized higher order chromatin state that defines heterochromatin and represses gene activity.

Other key factors for heterochromatin formation and gene silencing have been discovered. Many of the enzymes that remove histone modification marks associated with gene activity, or add marks associated with gene silencing, give Su(var) phenotypes when the gene is mutant, suggesting a progressive shift to the heterochromatic state [13]. Further, there is evidence that an ubiquitylation pathway is involved in H3-K9 methylation [19]. It has been recently proposed that mammalian gene silencing is also mediated by the interaction of HP1 with DNA methyltransferase 1 (DNMT1) [20•]. One of the most significant advances in the heterochromatin field has been the suggestion that RNA interference mechanisms are involved in heterochromatin formation, depending on the transcription of heterochromatic repeated sequences [12,21–23].

Telomeric functions of HP1

Cytogenetic studies have shown that HP1 is a stable component of all telomeres in *Drosophila*, including the

Figure 3



HP1 is involved in the control of telomere stability and telomere elongation. **(a)** Wild-type male metaphase (the numbers indicate the different autosome pairs while the X and Y symbols, respectively, indicate the X and Y sex chromosomes). **(b)** Male metaphase showing double telomeric fusions that involve the autosome pairs. **(c)** Single and multiple chromosome ring configurations. **(d)** DAPI stained polytene chromosomes from a larva obtained by crossing a female from a strain carrying the HP1 mutation *Su(var)2-5*^{04} with an *Ore-R* wild-type male. The telomere from the strain carrying the HP1 mutation (arrowhead) is clearly elongated in comparison to the telomere from the wild-type strain (arrow).

ends of stable terminal deletions lacking the telomeric transposons [24•]. Mutations in HP1 cause multiple telomere–telomere fusions in mutant cells, giving a striking spectrum of abnormal metaphase configurations [24•] (Figure 3). Telomeric fusions produce chromosome bridges during anaphase causing extensive chromosome breakage. This chromosome breakage means that the telomeric attachments are DNA end fusions rather than proteinaceous bridges and that the chromosome breakage initiates a BFB (breakage-fusion-bridge) cycle. These observations indicate that HP1 is a 'cap' protein essential for telomere stability, and its localization is independent of the sequences at the chromosome termini [24•]. Mutations in the chromodomain do not affect HP1 telomere localization and telomere stability, implying that HP1's telomeric position does not depend on an interaction with histone H3 trimethylated at lysine 9 (H3-Me3K9), although this modified histone is present at *Drosophila* telomeres [25]. ChIP and gel shift experiments have shown that HP1 directly binds telomeric DNA, independent of specific sequences, at the hinge region [26].

HP1 is involved not only in the capping function, but also in the replicative end function. In heterozygous HP1 mutant stocks, the telomeres elongate markedly over time, and the transcription of both TART and HeT-A is significantly increased [26,27]. In larvae lacking HP1, or carrying a mutation that disrupts the chromodomain, the transcription of the telomeric transposons is much more abundant [26]. Intriguingly, a functional chromodomain is also necessary for H3-K9 methylation at the telomeres [26]. Thus, a functional HP1 chromodomain and H3-Me3K9, although dispensable for telomere stability, are necessary for the correct transcription of telomeric transposons and for correct telomere elongation.

The role of HP1 at the telomeres is mediated by two different types of binding. Telomere capping depends on the direct binding of HP1 to telomeric sequences using the hinge domain, while the transcriptional control of telomeric sequences depends on the interaction of the HP1 chromodomain with H3-Me3K9. The observation that the H3-K9 methylation depends on the presence of HP1 at the telomeres suggests that this histone modification is due to an interaction of HP1 with a specific HMTase yet to be identified.

Interestingly, several recent findings have shown that HP1 is also involved in telomere metabolism in mammals. First, there is evidence for a telomeric localization of the different HP1 homologues on mammalian metaphase chromosomes [28–31]. Second, the HP1α homologue specifically interacts with Ku70, a protein that plays an important role in the maintenance and regulation of mammalian telomeres [32]. Third, overexpression of the HP1α and HP1β homologues in human cells, among several other effects, alters the telomeric association of the catalytic unit of telomerase (hTERT) and causes telomeric fusions. Fourth, the overexpression of all three HP1 homologues causes the shortening of 3′ overhang and the telomere size [33]. Finally, in mice the reduction of the chromobox proteins Cbx1, Cbx3 and Cbx5, which are most similar to *Drosophila* HP1a, is associated with an abnormal telomere elongation [31], similar to that observed in *Drosophila*.

HP1 is involved in positive regulation of gene expression

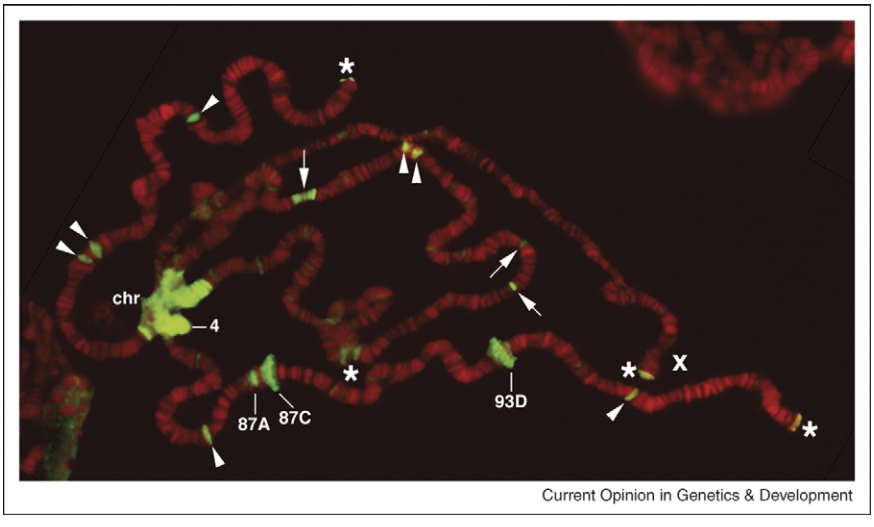
HP1 is present in some euchromatic regions of polytene salivary gland chromosomes in *Drosophila*, suggesting that HP1 also plays a role in the repression of specific subsets of euchromatic genes. This possibility has been supported by the finding that HP1 is involved in the

repression of four genes located in the cytological euchromatic region 31 of chromosome 2 where this protein is located [34]. However, an extensive cytoimmunochemical analysis in several different natural populations of *D. melanogaster* and other *Drosophila* species [8•] has shown that HP1 is located at about 200 euchromatic loci in a pattern that only partially overlaps with the euchromatic immunopattern produced by antibodies against H3meK9 [25]. These observations have suggested that the association of HP1 with euchromatic regions does not require an interaction with H3meK9. It has also been shown that there is not a strict correlation between HP1 euchromatic binding sites and the presence of transposon-like middle repetitive DNA [8•]. Thus, while HP1 may play a repressive role at some euchromatic sites, this seems unlikely to be the case at all such sites.

In fact, among its numerous euchromatic binding sites, HP1 associates with developmentally regulated chromosome puffs, structures that represent the visible expression of an intense gene activity at the chromosomal level. During the late third instar larval and prepupal stages, the release of the hormone ecdysone into the hemolymph induces a sequence of puffing activity that involves many loci. For example, in the polytene chromosomes in Figure 2a, three prominent ecdysone-induced puffs (75B, 74F, 71CD) are clearly decorated by the HP1 antibody. This association is particularly suggestive of an involvement of HP1 in ecdysone-induced gene activity. In support of this possibility, HP1 mutant larvae carrying the lethal allele *Su(var)2-5*^{02}, one that does not induce telomeric fusion or cell death, do not pupate and show a very long third instar (about 7–8 days) before they die. A detailed analysis of the heat-shock-induced expression of the HSP70 encoding gene in larvae either lacking HP1 or with an overdose of the protein has shown that HP1 is positively involved in *Hsp70* gene activity [35•]. A ChIP assay shows that HP1 binds the coding regions and not the promoter of the gene. The association of HP1 with the heat-shock-induced puffs is concomitant with the removal of the protein from almost all other euchromatic sites [35•] (see also Figure 4). Since it is well known that after the induction of heat-shock loci the rest of the genome is almost completely shut down [36], this observation has suggested a positive involvement of HP1 in euchromatic gene expression at a subset of loci. *In vivo* experiments on salivary glands, using RNAse treatment, or sodium salicylate treatment to induce the formation of puffs without transcription, have shown that the association of HP1 with euchromatic sites depends on the presence of RNA.

Taken together, these results strongly suggest that HP1 is positively involved in the expression of many euchromatic genes by an association with the corresponding transcripts [35•]. The results of numerous other of experiments lend support to this view. Many genes located in euchromatin in *Drosophila* are downregulated in mutant larvae lacking HP1. The analysis of some of these genes by simultaneous immunostaining with HP1 antibody and FISH with the corresponding probes, along with a ChIP assay, have shown that these genes are associated with HP1 [37•]. These results have unequivocally shown that HP1 is involved in positive regulation of euchromatic

Figure 4



The HP1 immunopattern on polytene chromosomes from a heat shock treated larva. A comparison with the HP1 immunopattern on untreated polytenes reported in Figure 2a, reveals that, concomitantly with the HP1 accumulation on heat-shock-induced puffs (87A, 87C, 93D and others indicated by the arrowheads), many of the other euchromatic signals disappear or appear very faint with the exception of those located on the 31 region and few others (arrows). The immunofluorescence on the telomeres (asterisks), the chromocenter (chr) and the fourth chromosome (4) appears unchanged.

gene expression at a subset of loci. Supporting results have been obtained by depletion of HP1 in cultured cells [38]. More recently, high-resolution mapping experiments have also shown that HP1 is associated with transcriptionally active chromatin in *Drosophila* [39•,40]. Experiments in mammalian systems also point to the involvement of HP1 in gene expression [41]. Intriguingly, a recent study of the *Drosophila* PIWI protein has suggested a possible role of noncoding RNA in targeting HP1. PIWI belongs to the ARGONAUTE/PIWI family and binds the PIWI-interacting RNAs (piRNAs). It has been shown that PIWI interacts with HP1 and displays an overlapping (but not congruent) distribution pattern on polytene chromosomes, including both heterochromatic and euchromatic sites. The PIWI distribution pattern also depends on RNAse [42•].

Conclusions

Even from this brief discussion, the role of HP1 clearly emerges as that of an adaptor involved in different functions regarding chromatin configuration. Other properties and functions not discussed here – such as the interaction of HP1 with nuclear membrane, its role in metaphase chromatid cohesion and centromere organization and its involvement in human diseases such as cancer – await more intense study. We think HP1 will disclose other intriguing features in the future. The main problem will be to understand if the functional versatility of HP1 depends on a unique mode of action (i.e. it performs the same activity with different partners in different contexts) or if it possesses several modes of action. These could depend on conformational changes due to post-translational modifications that in turn arise from an epigenetic subcode that permits different interactions in different contexts [10•].

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