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13. ABSTRACT (Maximum 200 Words)

Metastasis accounts for the majority of deaths associated with breast cancer. There is a need to identify prognostic/predictive indicators to accurately determine whether breast cancer cells are likely to metastasize. $HP1^{Hs\alpha}$ (Heterochromatin Protein 1) has been identified as a candidate breast cancer metastasis suppressor that is down-regulated in highly invasive/metastatic breast cancer cells compared to poorly invasive/non-metastatic cells. $HP1^{Hs\alpha}$ is a non-histone chromosome protein that plays a role in chromosome segregation, chromatin packaging, and gene silencing. Two approaches have been taken to modulate levels of $HP1^{Hs\alpha}$ in breast cancer cells. First, an adenovirus construct has been generated to express $HP1^{Hs\alpha}$ in invasive/metastatic breast cancer cells. Second, RNAi is being used to knock-down $HP1^{Hs\alpha}$ levels in poorly invasive/non-metastatic cells. Levels of $HP1^{Hs\alpha}$ expression will be correlated with alterations in gene expression and invasive properties of these cells. Down-regulation of the $HP1^{Hs\alpha}$ gene in highly invasive/metastatic breast cancer cells does not correlate with alterations in methylation of the CpG island within the $HP1^{Hs\alpha}$ promoter region. A150 bp promoter fragment has been identified that confers differential regulation in highly invasive/metastatic and poorly invasive cells/non-metastatic cells. Thus, a regulatory factor acting through this region is likely to be responsible for the differential regulation.

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INTRODUCTION

Breast cancer accounts for approximately 30% of all diagnosed cancer and is the most common malignancy in women. The lethality of breast cancer is mainly attributed to its ability to metastasize throughout the body. The five year survival rate for individuals with metastatic breast cancer is 21%, in contrast to 95% for those with non-metastatic breast cancer. Although there has been significant progress made in the identification and understanding of breast cancer over recent years, there remains a need for the identification of molecular markers that definitively distinguish poorly invasive/non-metastatic tumors from highly invasive/metastatic tumors. To identify such prognostic/predictive markers, a clearer understanding of the progression of the metastatic disease state is required.

We have identified a molecular marker that is a candidate breast cancer metastasis suppressor. This marker, termed Heterochromatin Protein 1 ($HP1^{Hs\alpha}$), is an evolutionarily conserved non-histone chromosomal protein (3). HP1 primarily localizes to centric heterochromatin where it plays a role in chromosome segregation and silencing of genes brought into juxtaposition with heterochromatin (11). HP1 also localizes within the gene-rich euchromatic regions of the genome where it is proposed to play a role in gene regulation (8, 10). We discovered that $HP1^{Hs\alpha}$, one of three HP1 proteins in humans, was significantly down-regulated in highly invasive/metastatic breast cancer cells compared with poorly invasive/non-metastatic breast cancer cells (6). [This observation was specific for $HP1^{Hs\alpha}$, and not the other two HP1 family members.] We discovered a similar correlation with $HP1^{Hs\alpha}$ levels in breast cancer patients: $HP1^{Hs\alpha}$ was abundant in the nuclei of cells from primary breast tumors, but dramatically reduced in cells of metastatic tissue (6). When highly invasive/metastatic breast cancer cells were transfected with an $HP1^{Hs\alpha}$ transgene they showed a reduction in *in vitro* invasion {Kirschmann, 2000 #96. Our working model is that $HP1^{Hs\alpha}$ regulates genes involved in invasion and metastasis in breast cancer cells.

BODY

Each task and progress on that task is discussed below. All tasks are identical to those stated in the approved STATEMENT OF WORK in the original proposal.

Task 1:Determine the consequences of $HP1^{Hs\alpha}$ expression on tumor metastasis markers and global transcriptional expression in human breast cancer cells.

This task is based on the observation that poorly invasive/non-metastatic breast cancer cells (MCF-7) have levels of $HP1^{Hs\alpha}$ similar to most cell types, whereas, highly invasive/metastatic cells (MDA-MB-231) have low levels of $HP1^{Hs\alpha}$. We generated stably transfected MDA-MB-231 cells that expressed $HP1^{Hs\alpha}$. Unfortunately, over time the transfected cell population shut down expression of the $HP1^{Hs\alpha}$ transgene, making it impossible to obtain reproducible data on molecular markers, cell morphology and gene expression changes. Furthermore, it was not possible to clone the transfected cells; numerious trials resulted in cell death. Therefore, we took an alternate approach to

deliver HP1$^{Hs\alpha}$ to MDA-MB-231 cells. We generated an adenoviral vector containing a GFP-tagged version of HP1$^{Hs\alpha}$. We have shown that the GFP-tagged version rescues the lethality of a Drosophila HP1 mutant, demonstrating that the fusion protein is likely to be functional. We have infected cells with the adenoviral vector and observed GFP fluorescence concentrated in the nucleus, showing the centric staining pattern virtually identical to that of endogenous HP1. We are now in the final process of generating the control adenoviral vector containing a nuclear-targeted GFP. Once complete, comparative studies on the biological effects of expressing GFP-HP1 and GFP alone will be performed as outlined in the original Task 1.

As a counter experiment to the HP1$^{Hs\alpha}$ expression studies, a reviewer of our grant proposal suggested that we perform RNAi experiments to knock-down the levels of HP1$^{Hs\alpha}$ in poorly invasive/non-metastatic cells. At the time when we submitted the proposal, the prospects of RNAi working in mammalian cell culture were not good so we did not propose to do so. Since that time, there has been significant progress made in RNAi technology in the mammalian cell culture field {Paddison, 2002 #160}. Therefore, we have constructed four RNAi plasmid constructs directed to different regions within the *HP1$^{Hs\alpha}$* mRNA. The use of plasmid-based RNAi constructs will allow for the recovery of stable lines expressing the interfering RNA. All constructs are being co-transfected with a plasmid containing GFP. Transfected cells are sorted for GFP fluorescence and levels of HP1$^{Hs\alpha}$ in transfected cells are being analyzed by RT-PCR and Western blot analysis. As a complementary approach, an siRNA pool (Dharmacon) has been purchased and will be transfected into MCF-7 cells. If successful, HP1$^{Hs\alpha}$ levels will be transiently knocked down within MCF-7 cells. Based on the HP1-dosage sensitivity in Drosophila, it is likely that gene expression changes will occur even with a transient knock-down of HP1$^{Hs\alpha}$ levels. Therefore, much of the biological assays proposed under Task 1 will be feasible.

Task 2: Determine the molecular mechanisms of HP1$^{Hs\alpha}$ down-regulation in human breast cancer invasion/metastasis.

The rationale for this task is that HP1$^{Hs\alpha}$ mRNA and protein levels in MDA-MB-231 cells are reduced to approximately 20% that of MCF-7 cells. We inferred that a component of the down-regulation might be at the transcription level. We generated five constructs containing varying amounts (100 to 600 bp) of *HP1$^{Hs\alpha}$* promoter region fused to *luciferase* (Figure 1). These constructs were transfected into MCF-7 and MDA-MB-231 breast cancer cells and luciferase measurements were performed. The results demonstrate that promoter activity is present in all constructs and that the differential expression of endogenous *HP1$^{Hs\alpha}$* between the two cells types appears to be due sequences residing 150 bp upstream of the *HP1$^{Hs\alpha}$* transcription start site. The experiments will be repeated in two different cell lines, ZR-75-1 and Hs578T, which have similar invasive/metastatic properties as MCF-7 and MDA-MB-231, respectively.

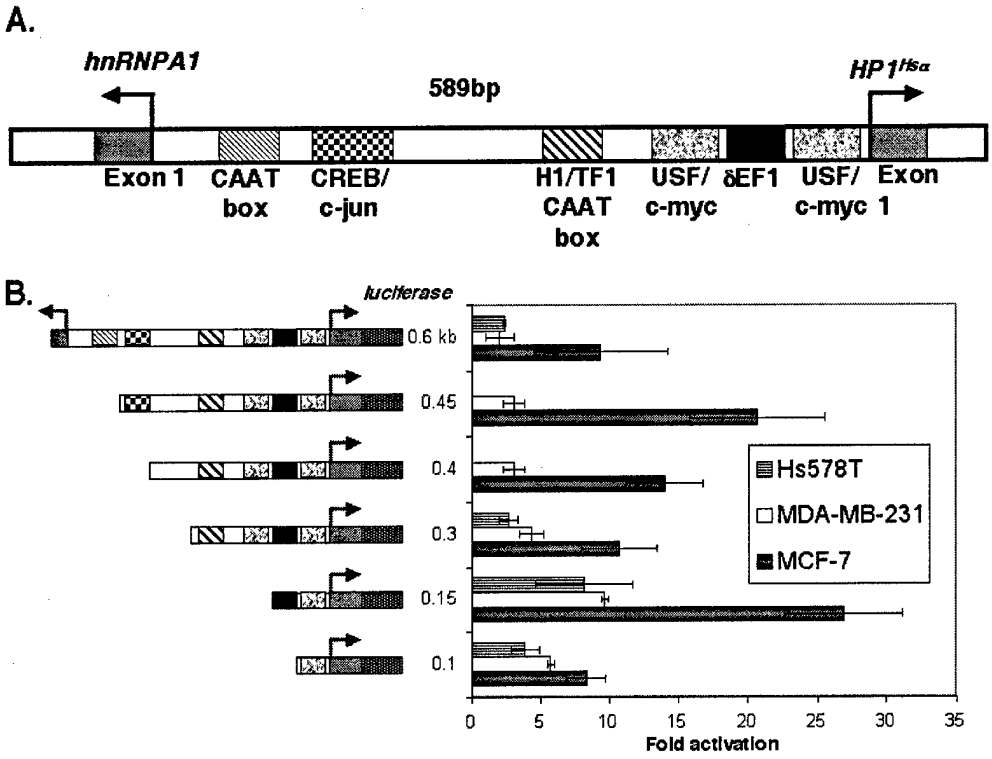

Figure 1. Differential *HP1*$^{Hs\alpha}$ promoter expression driving the *luciferase* reporter gene. (**A**) Diagrammatic representation of the *HP1*$^{Hs\alpha}$ promoter region. Divergent transcription start of *HP1*$^{Hs\alpha}$ and *HnRNP A1* are 589 bp upstream from each other. Several transcription factor binding sites were identified by MatInspector analysis and footprinting assays (1) that do not affect expression of *HnRNP A1*. (**B**) Differential expression of a *luciferase* reporter gene from various deletions and mutant constructs of the *HP1*$^{Hs\alpha}$ promoter. MCF-7, a poorly invasive, nonmetastatic breast cancer cell lines has significantly higher fold expression of *luciferase* from the *HP1*$^{Hs\alpha}$ promoter constructs than MDA-MB-231 and Hs578T, both highly invasive and highly metastatic breast cancer cell lines.

A bioinformatics analysis of the *HP1*$^{Hs\alpha}$ promoter sequences determined that a δEF1 binding site was present within the 150 bp region that confers differential expression. Interestingly, AREB6, a zinc finger transcriptional repressor that binds to this site (4), has been shown to be up-regulated in MD-MB-231 cells (7). Therefore, we postulate that up-regulation of AREB6, hence greater occupancy at the *HP1*$^{Hs\alpha}$ promoter, might be responsible for *HP1*$^{Hs\alpha}$ transcriptional down-regulation in highly invasive/metastatic breast cancer cells. Constructs that delete and mutate the δEF1 binding site are currently being generated to test this hypothesis. We are also generating constructs in which the 150 bp promoter fragment is linked to a heterologous promoter to

determine if it is sufficient for the differential expression. These results are currently being prepared for publication.

A second possibility to explain the differential expression of *HP1*$^{Hs\alpha}$ in invasive/metastatic cells and poorly invasive/non-metastatic cells is promoter methylation. *HP1*$^{Hs\alpha}$ has a CpG island upstream of transcription start. In collaboration with Michael Kladde (Texas A&M University), we discovered that the promoter region of *HP1*$^{Hs\alpha}$ is hypomethylated in both MCF-7 and MDA-MB-231 cells (figure 2). Thus, differential methylation cannot account for the down-regulation.

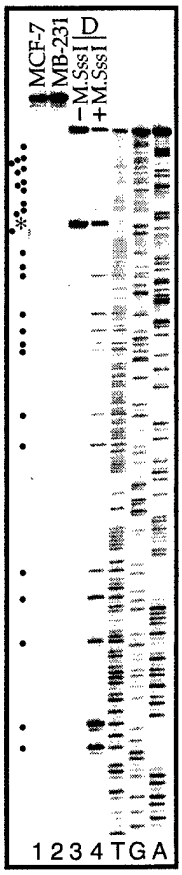

Figure 2. Absence of detectable 5-methylcytosine at the *HP1*$^{Hs\alpha}$ promoter in MCF-7 and MDA-MB-231 cells. DNA from both cell lines (lanes 1 and 2) was subjected to a sensitive variation of genomic bisulfite sequencing that is able to detect low levels of ^{5me}C. Sequences from -220 to +168 of the HP1$^{Hs\alpha}$ promoter are shown. Plasmid DNA containing the HP1$^{Hs\alpha}$ promoter methylated *in vitro* by M.*SssI* (lane 4), provided a marker for modified CpG sites (filled circles). Since the plasmid was isolated from a *dcm*+ strain of *E.coli,* methylation at a *dcm* site (asterisked) was also detected (lanes 3 and 4). Methylation at the *dcm*- and M.*SssI*-modified sites demonstrates the signal that is commensurate with high and moderate levels of DNA methylation, respectively. Thus, MCF-7 and MDA-MB-231 cells do not have detectable levels of ^{5me}C in the *HP1*$^{Hs\alpha}$ promoter region.

Task 3: Determine the domains of HP1$^{Hs\alpha}$ required for invasion and metastasis.

HP1$^{Hs\alpha}$ has a two domain structure consisting of an amino chromo domain and a carboxy chromo shadow domain. The chromo domain associates with methylated lysine 9 of histone H3 (5) and is thought to be the primary mechanism of HP1 localization within centric heterochromatin. Recent data suggest that HP1 localization at other genomic locations is likely to occur through alternative mechanisms (2, 9). In order to determine what domains are involved in governing the metastatic potential of a breast cancer cell, we have generated four constructs. These include a truncation, leaving just the chromo domain, a truncation that leaves just the chromo shadow domain, a point mutation in the chromo domain that has been shown to disrupt the interaction with methylated lysine 9 of histone H3, and a point mutation that has been shown to disrupt HP1 dimerization. These

constructs are being cloned into adenoviral vectors and will be used to analyze the phenotypes discussed in Task 1.

KEY RESEARCH ACCOMPLISHEMENTS

- Generated adenoviral vectors that express GFP- HP1$^{Hs\alpha}$ and a nuclear targeted GFP. These will be used to infect highly invasive/metastatic breast cancer cells and determine the biological effects of HP1$^{Hs\alpha}$ expression.
- Generated four RNAi plasmid constructs that will be used to knock-down HP1$^{Hs\alpha}$ levels in poorly invasive/non-metastatic breast cancer cells.
- Identified candidate regulatory factor binding sites upstream of the *HP1$^{Hs\alpha}$* transcription start site.
- Determined the *HP1$^{Hs\alpha}$* promoter region necessary for expression.
- Identified a 150 bp fragment that is necessary for differential expression between highly invasive/metastatic and poorly invasive/non-metastatic breast cancer cells.
- Determined that *HP1$^{Hs\alpha}$* was not differentially methylated in highly invasive/metastatic breast cancer cells compared with poorly invasive/non-metastatic breast cancer cells.
- Generated plasmid constructs containing portions of the HP1$^{Hs\alpha}$ coding region.

REPORTABLE OUTCOMES

Publication

Li, Yuhong, Kirschmann, D.A., L. L. Wallrath. (2002). Does heterochromatin protein 1 always follow code? Proc. Natl. Acad. Sci. USA. 99: 16462-16469.

Poster Abstract

Norwood, L.A., Kirschmann, D.A., Grade, S.K., Hines, K.A., Cryderman, D.E., Toro, R., Seftor, E.A., Hendrix, M.J.C., L.L. Wallrath (2002). HP1$^{Hs\alpha}$ and breast cancer metastasis. 2nd International Conference on Prostate Cancer Research, University of Iowa, Iowa City, IA.

CONCLUSIONS

We have spent the better part of this year developing tools to modulate the levels of in *HP1$^{Hs\alpha}$* breast cancer cells. These tools are now in place and the biological consequences of modulating *HP1$^{Hs\alpha}$* will be investigated. We have made significant progress in identifying the genomic region of *HP1$^{Hs\alpha}$* required for expression and have discovered that the differential expression between the two different types of breast cancer cells resides in a 150 bp fragment. Furthermore, differential expression does not appear to be governed by changes in DNA methylation. These results suggest that the 150 bp element might associate with a factor that is differentially expressed in highly invasive/metastatic breast cancer cells versus poorly invasive/non-metastatic breast cancer cells.

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APPENDICES

1. One Publication
2. One Poster Abstract

Does heterochromatin protein 1 always follow code?

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Heterochromatin protein 1 (HP1) is a conserved chromosomal protein that participates in chromatin packaging and gene silencing. A loss of HP1 leads to lethality in *Drosophila* and correlates with metastasis in human breast cancer cells. On *Drosophila* polytene chromosomes HP1 is localized to centric regions, telomeric regions, in a banded pattern along the fourth chromosome, and at many sites scattered throughout the euchromatic arms. Recently, one mechanism of HP1 chromosome association was revealed; the amino-terminal chromo domain of HP1 interacts with methylated lysine nine of histone H3, consistent with the histone code hypothesis. Compelling data support this mechanism of HP1 association at centric regions. Is this the only mechanism by which HP1 associates with chromosomes? Interest is now shifting toward the role of HP1 within euchromatic domains. Accumulating evidence in *Drosophila* and mammals suggests that HP1 associates with chromosomes through interactions with nonhistone chromosomal proteins at locations other than centric heterochromatin. Does HP1 play a similar role in chromatin packaging and gene regulation at these sites as it does in centric heterochromatin? Does HP1 associate with the same proteins at these sites as it does in centric heterochromatin? A first step toward answering these questions is the identification of sequences associated with HP1 within euchromatic domains. Such sequences are likely to include HP1 "target genes" whose discovery will aid in our understanding of HP1 lethality in *Drosophila* and metastasis of breast cancer cells.

In eukaryotes, there are two major types of chromatin: heterochromatin and euchromatin (1). Heterochromatin corresponds to the relatively gene-poor, late-replicating, repetitious sequences found near centric and telomeric locations. In contrast, euchromatin replicates relatively early in the cell cycle and contains single copy sequences, including the majority of genes. Both euchromatin and heterochromatin are packaged into nucleosomes, the fundamental packaging unit consisting of a histone octamer. Euchromatin and heterochromatin can be distinguished by specific histone tail modifications. In general, the histone tails in heterochromatin are relatively hypoacetylated; however, acetylation of lysine twelve of histone H4 is a distinguishing mark for heterochromatin (2–4). In contrast, histone H3 and H4 tails found in euchromatin are generally acetylated (4). Histone H3 acetylation is often linked to H3 phosphorylation and is likely to represent a two-component code for high levels of gene expression (5, 6).

In addition to distinct differences in histone modification, euchromatin and heterochromatin show differences in nonhistone chromosomal protein constituents. One of the best-studied examples is heterochromatin protein 1 (HP1) first discovered in *Drosophila* and named for its predominant localization to centric heterochromatin (7) (Fig. 1*A*). Consistent with this localization, the gene encoding HP1, *Su(var)2–5*, was isolated as a dominant suppressor of position effect variegation (PEV) (8, 9). PEV is the mosaic pattern of expression exhibited by genes placed near centric heterochromatin by chromosomal rearrangements or transposition events (10). Overexpression of HP1 leads to enhanced silencing of variegating genes. Conversely, a decreased level of HP1 leads to reduced silencing of variegating genes. A complete loss of HP1, as in homozygous *Su(var)2–5* null mutants, results in lethality. Larvae survive until the late third instar stage because of maternally supplied HP1 (11, 12). The cause of lethality is unknown. Given the centric localization of HP1, and the interaction between the *Schizosaccharomyces pombe* HP1-like protein Swi6 and a cohesion protein, chromosome segregation might be affected (13, 14). Thus, HP1 levels are critical for regulating the extent of heterochromatization within centric regions that is required for proper chromosome segregation.

In addition to centric regions, HP1 is observed at other regions of the genome known to be heterochromatic. The small fourth chromosome of *Drosophila melanogaster*, interspersed with heterochromatic domains, shows a banded pattern of HP1 localization (7, 15). Consistent with HP1 having a packaging function at these locations, transgenes inserted along the fourth chromosome exhibit PEV that is suppressed by *Su(var)2–5* mutations (15, 16). HP1 localization is also observed at *Drosophila* telomeres that terminate in repetitive arrays of retrotransposons (17). Telomeric association, however, appears to be independent of primary DNA sequence as broken chromosomes lacking terminal retrotransposons retain HP1 association (12). Telomere-telomere fusions occur in larval neuralblasts of *Su(var)2–5* mutants, suggesting HP1 plays a role in telomere capping (12).

In contrast to these chromosomal domains rich in repetitive DNA sequences, HP1 is present at approximately 200 sites within the euchromatic arms of polytene chromosomes that are relatively poor in repetitious DNA sequences. Do these sites represent small domains of repressive chromatin? Are there genes at these sites that are regulated by HP1? These questions are currently under investigation.

Here we describe current studies on the role of HP1 in gene regulation at both euchromatic and heterochromatic domains. We summarize the results from reports that have identified HP1 partner proteins and discuss implications for these findings. Last, we hypothesize about multiple mechanisms of HP1 chromosome association and their impact on gene expression.

HP1 Follows Code

HP1 is a highly conserved protein with family members found in a variety of eukaryotic organisms ranging from *S. pombe* to humans (18–21). In *Drosophila*, two additional HP1-like proteins, HP1b and HP1c, sharing amino acid sequence similarity and domain structure, have been identified (22) (Fig. 2). Whereas HP1b shows localization to both euchromatin and heterochromatin just as HP1, HP1c is found only in euchromatin (22). Mice and humans each have three HP1-like proteins that possess similarities in amino acid sequence, domain structure,

This paper results from the Arthur M. Sackler Colloquium of the National Academy of Sciences, "Self-Perpetuating Structural States in Biology, Disease, and Genetics," held March 22–24, 2002, at the National Academy of Sciences in Washington, DC.

Abbreviations: CAF1, chromatin assembly factor 1; CD, chromo domain; CSD, chromo shadow domain; HP1, heterochromatin protein 1; ORC, origin recognition complex; HOAP, HP1/ORC-associated protein; PEV, position effect variegation; Rb, retinoblastoma.

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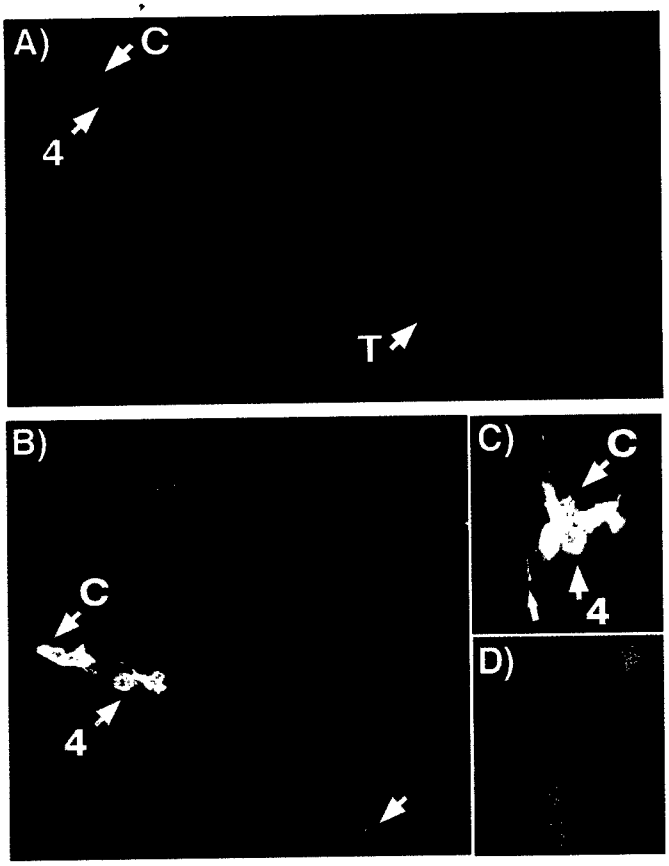


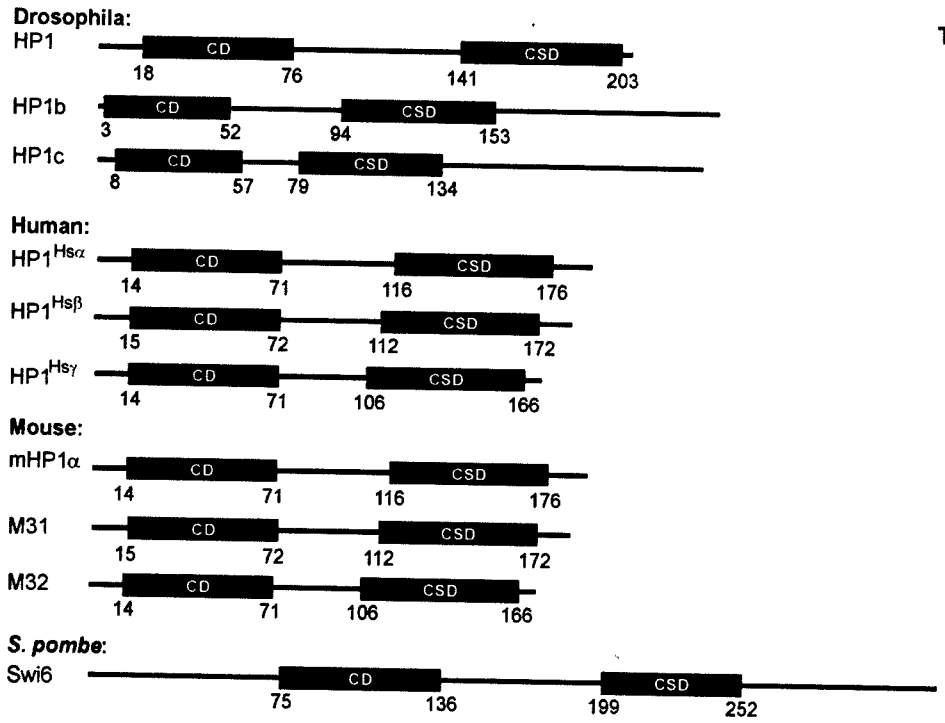
Fig. 1. (*A*) Pattern of HP1 distribution on *Drosophila* polytene chromosomes. *D. melanogaster* larval polytene chromosomes were stained with mouse monoclonal C1A9 antibodies against HP1 (gift of Sarah C. R. Elgin) and a secondary antibody conjugated with rhodamine. The chromocenter (C), the fourth chromosome (indicated by 4), telomeres (T), and euchromatic sites associated with HP1. (*B*) The pattern of HP1 and methylated lysine nine of histone H3 on *Drosophila* polytene chromosomes. *D. melanogaster* larval polytene chromosomes were stained with mouse monoclonal C1A9 antibody against HP1 and a rabbit polyclonal antibody that recognizes methylated lysine nine of histone H3 (gift of C. David Allis, University of Virginia, Charlottesville). A Cy5-conjugated rabbit secondary antibody and a FITC-conjugated mouse secondary antibody were used for detection. The chromocenter (C) and the fourth chromosome (indicated by 4) show strong colocalization (yellow). Example locations enriched in HP1 are denoted by green arrows; example locations enriched in methylated lysine nine of histone H3 are indicated by red arrows. (*C*) Same as in *B*, showing a closer view of the chromocenter region. (*D*) Same as in *B*, showing a closer view of a telomeric region.

and centric chromosomal localization properties as *Drosophila* HP1 (Fig. 2). Although there are minor differences in chromosomal localization and protein interaction partners for HP1-like proteins within a given species (23), it is not clear whether these proteins have specific or redundant functions. In flies, mice, and humans, the HP1-like proteins are small in size, ranging from 173 to 240 aa (Fig. 2). Overall the percent identity of HP1-like proteins to *Drosophila* HP1 is approximately 50% for mammalian HP1-like proteins. The majority of conserved amino acids are concentrated in two domains. The structure of HP1-like proteins can be simplified as two conserved domains separated by a less conserved hinge region (Fig. 2). The conserved amino-terminal region of HP1-like proteins is termed the chromo domain (CD) (24). This domain is present in 20 proteins in Flybase (www.ebi.ac.uk/proteome/DROME/interpro/stat.html), many of which play roles in gene regulation. The conserved carboxyl-terminal region, termed the chromo shadow domain (CSD), is related to the CD in primary amino acid sequence (25). Both the CD and the CSD have been the subject of extensive structural analysis (26–30). Each domain forms a hydrophobic pocket. The CSD dimerizes (18, 27, 28, 31) as well as interacts with a wide variety of nuclear proteins (see below). Cross-species functional studies in which the mouse HP1-like protein M31 was expressed in *S. pombe* indicate that species-specific functions of HP1 reside within the CSD (32). The CD is required for chromosome association (33).

The mechanism(s) by which HP1 establishes the complex localization pattern on chromosomes remained a mystery for over a decade since its discovery. For many chromosomal proteins, localization is achieved through direct interaction with DNA sequences. Attempts to identify specific interactions between HP1 and DNA sequences, particularly repetitive DNA sequences found within heterochromatin, were not particularly revealing (34). For some chromosomal proteins, localization is achieved through interactions with DNA binding proteins. Therefore, a search for HP1 partner proteins might reveal the "missing link" between HP1 and the chromosome. A phage display assay was performed to identify peptides that interact with the CD and CSD (31). This assay revealed peptide sequences that showed a specific interaction with the CSD. Comparison of the peptide sequences allowed a consensus pentapeptide to be generated (31). Supporting these results, the consensus pentapeptide was found in several proteins shown to interact with HP1 by other types of assays (35–37). To date, the localization pattern of candidate interacting proteins cannot explain the entire localization pattern observed for HP1. In contrast to the results obtained for the CSD, no peptides were identified from the phage display assay that specifically interacted with the CD. These results were surprising because a point mutation within the CD of *Drosophila* HP1 eliminates the majority of chromosome association, suppresses PEV, and is homozygous lethal (9).

The mystery surrounding interactions of the CD was solved by studies of the murine Suv39h1 protein, a homologue of the *Drosophila* SU(VAR)3–9 protein (38, 39). A comparative genomic approach in combination with biochemical studies revealed that the SET [a conserved motif in *Drosophila Su(rar)3-9*, *Enhancer of Zeste*, and *trithorax*] domain of Suv39h1 contains methyltransferase activity specific for lysine nine of histone H3. This methylation mark on the histone H3 tail serves as a specific recognition code for the CD of HP1. This discovery supports the histone code hypothesis that proposes histone tail modifications serve as specific recognition motifs for chromatin proteins (40). The HP1 CD, but not the CD of several other proteins, binds methylated lysine nine of histone H3 (41). Therefore, the substrate specificity is likely caused by minor differences in the amino acid sequences of CDs from different proteins. The connection between Suv39h1 and HP1 is consistent with *Drosophila* research showing that the genes encoding HP1 and SU(VAR)3–9 genetically interact with the heterochromatin silencing system (8) and that the proteins physically interact (42). The relationship between HP1 and Suv39h1 has been maintained by the *S. pombe* homologues, Swi6 and Clr4, respectively (43, 44), suggesting evolutionary conservation in this mechanism of chromosome association and heterochromatic gene silencing.

In summary, HP1 serves as a bridging protein, connecting histones, through interactions with the CD, to nonhistone chromosomal proteins, through interactions with the CSD (Fig. 3*A*). In this case, Suv39h1 sets the histone code for HP1 association. Based on these findings, mechanisms for heterochromatin spreading have been proposed to involve recruitment of Suv39h1 by HP1 and propagation of the methylation mark along the chromosome (39). Details of such spreading mechanisms remain to be elucidated.



	Total AA	% Identity Total	CD	Hinge	CSD
	206	100	100	100	100
	240	51	58	42	58
	237	44	53	33	36
	191	49	60	27	54
	185	54	67	26	56
	173	52	59	24	52
	191	49	60	27	54
	185	54	67	26	56
	173	52	59	24	52
	328	25	48	13	17

Fig. 2. Diagram of HP1 proteins in *Drosophila*, mouse, human, and *S. pombe*. Total length of each protein is indicated and drawn to relative scale. Percent identity when compared with *Drosophila* HP1 over the full length (total), or the CD, CSD, or the hinge region was calculated according to ref. 85.

Are There Multiple Mechanisms for HP1 Association?

HP1 localizes to distinctly different environments throughout the genome. Has the discovery of the interaction with the methylated lysine nine of histone H3 cracked the code, or are there alternative mechanisms of HP1 chromosomal association? The importance of this question is evident when reviewing data on the *Su(var)2–5*^{02} allele of the gene encoding HP1. This allele contains the amino acid substitution of a highly conserved valine to a methionine in the CD. Structural analysis indicates that this residue plays a critical role in the formation of the hydrophobic pocket of the CD (30). In most genetic silencing assays this allele behaves as an HP1 null; however, an important distinction between this allele and null alleles was revealed by a cytological analysis of HP1 staining on chromosomes from HP1 mutants. Whereas null alleles show no HP1 chromosome association, the *Su(var)2–5*^{02} allele shows diminished HP1 localization to centric regions, but retains association at euchromatic and telomeric sites (12). These data suggest an alternative mechanism of HP1 association might be operating at noncentric locations.

Further evidence for alternate mechanisms of association comes from cytological experiments on polytene chromosomes in wild-type flies. The pattern of staining observed by antibodies to HP1 and methylated lysine nine of histone H3 is not completely coincident. Both antibodies show colocalization to the chromocenter and along the fourth chromosome, but not throughout the euchromatic arms and at telomeric regions (Fig. 1 *B–D*) (45). One technical explanation for incomplete colocalization is that the epitopes recognized by either antibody are masked by fixation at specific genomic locations. However, if this is not the case, sites within the euchromatic arms that stain with only the HP1 antibody could be generated by HP1 associations through mechanisms independent of SU(VAR)3–9. Interactions of HP1 with unmodified histone tails, the histone-fold domain, and histone H1 might account for the staining pattern observed (34, 46). Such possibilities are diagrammed in Fig. 3*B*. Alternatively, interactions with nonhistone chromosomal proteins might serve as an additional mechanism of association (Fig. 3*C*). Interactions between HP1 and transcriptional corepressors that associate with DNA binding proteins (see below) (47, 48) support this hypothesis. The double staining also revealed sites within the euchromatic arms that are detected only by the methyl lysine nine histone H3 antibody. These sites could correspond to different degrees of methylation because the antibody recognizes only dimethylated lysine (Upstate Biotechnology, Lake Placid, NY); HP1 is thought to recognize both methylated states with relatively equal affinity (30). Another explanation for lack of complete colocalization of HP1 and the methyl lysine nine histone H3 antibody is that additional histone modifications might be present that do not permit HP1 association (Fig. 3*D*). Clearly the code for chromosomal protein association might have multiple components.

HP1 Interacts with a Myriad of Proteins

Does the identification of proteins that associate with HP1 provide clues about the mechanisms of silencing? Genetic analysis of PEV in *Drosophila* provided a collection of mutations that encode candidate HP1 interaction partners: for example, SU(VAR)3–9, the histone methylase discussed above interacts with HP1 by two-hybrid analysis (42) (Table 1). A second example is SU(VAR)3–7, a zinc finger protein that associates with satellite DNA sequences (49). HP1 and SU(VAR)3–7 colocalize in the *Drosophila* embryo and on polytene chromosomes (50, 51) and interactions between the two proteins have been demonstrated by yeast two-hybrid analysis and coimmunoprecipitation from embryonic extracts (35, 50). More specifically, the CSD of HP1 interacts with multiple regions of SU(VAR)3–7, but it is not yet clear how these two proteins collaborate to form and/or spread heterochromatin.

In addition to a gene silencing function, HP1 is thought to play a role in nuclear organization. This hypothesis is based on the discovery that HP1 interacts with lamin B receptor, either directly (52, 53) or indirectly through interactions with histones

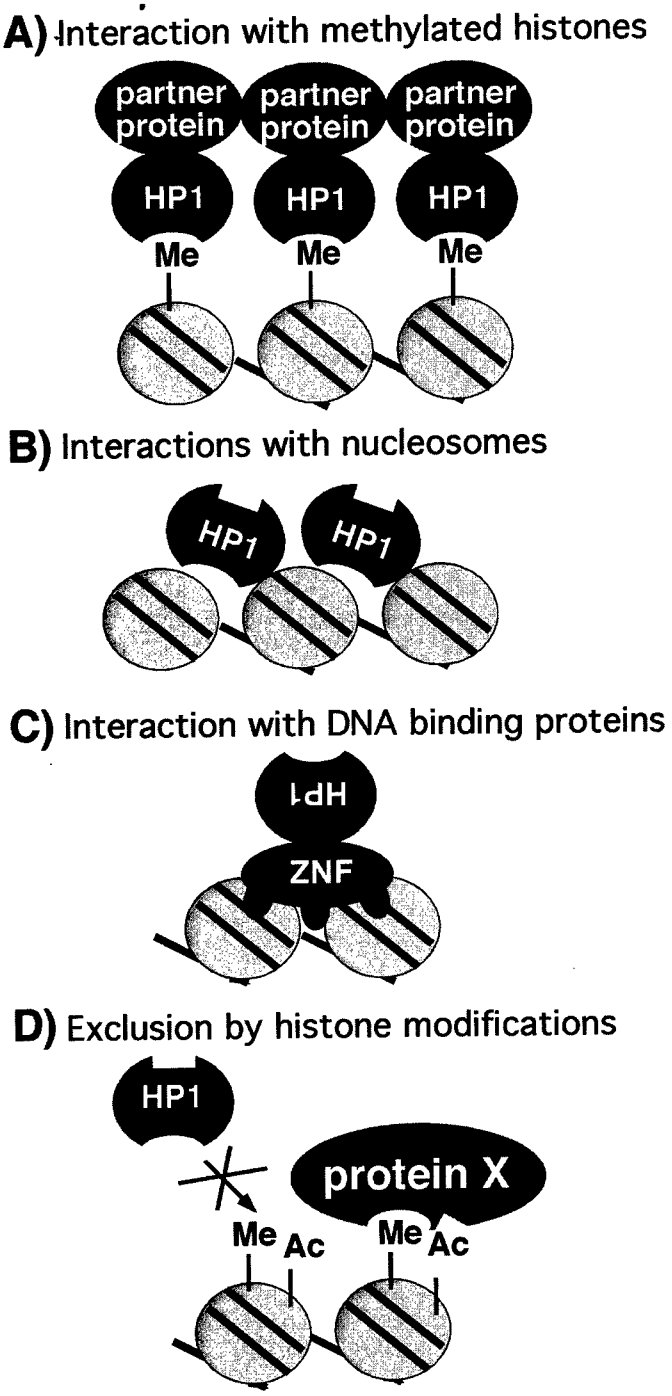


Fig. 3. Models for HP1 association and nonassociation with chromosomes. (*A*) Interaction between the HP1 CD with the methylated lysine nine of histone H3. HP1 serves as a bridge for partner proteins. (*B*) HP1 interacts with histones in a nonmethylated-dependent fashion. (*C*) HP1 associates with chromosomes through interactions of the CSD and DNA binding proteins, such as zinc-finger proteins (ZNF). (*D*) HP1 does not associate with methylated histones that have additional modifications such as acetylation or phosphorylation.

(54). In addition, experimental data support an interaction between HP1 and B-type lamin and Lap2β, lamin-associated protein, located within the nuclear envelope. *In vitro*, these interactions foster nuclear envelope assembly, suggesting HP1 plays a role in organizing nuclear architecture (55). Given that heterochromatin localizes to the nuclear periphery in many eukaryotic cell types, HP1 might tether heterochromatin to the nuclear envelope, leaving active regions of the genome free to coalesce into transcription factories within the interior of the nucleus (56).

The localization of HP1 to many sites throughout the *Drosophila* euchromatic arms brings to question the role of HP1 in the regulation of gene expression. Supporting a role for HP1 in transcriptional regulation, HP1 has been shown to interact with numerous proteins involved in modulating chromatin structure and gene expression (Table 1). In mammals, association of HP1, the retinoblastoma (Rb) protein and SUV39H1 with the *cyclin E* promoter correlates with gene silencing (57). Furthermore, HP1 has been implicated in gene repression mediated by Krüppel-associated box (KRAB) zinc finger proteins (47, 48). Taken together, these findings suggest that HP1 is recruited to specific genes by protein–protein interactions, resulting in gene silencing by an unknown mechanism.

In addition to transcriptional regulators, HP1 interacts with proteins involved in DNA replication and repair. Chromatin assembly factor 1 (CAF1) is a three-subunit complex that assembles histones H3 and H4 onto newly replicated DNA in both euchromatic and heterochromatic regions of the genome. In mammals, the large subunit, p150, contains a domain that interacts with the CSD of HP1 (37). Deletion of this domain does not alter CAF1-mediated chromatin assembly after replication *in vitro* or targeting of HP1 to heterochromatin *in vivo* during DNA replication. However, deletion of this domain reduces the amount of CAF1 present in heterochromatin outside of S phase. Although the significance of CAF1-HP1 interaction is not clear, the data suggest that CAF1 might stabilize heterochromatin structure during times of chromosome decondensation and transcription.

HP1 associates with origin recognition complex (ORC) proteins (58, 59). This presents an intriguing parallel to the situation in *Saccharomyces cerevisiae* where ORC proteins associate with silent information regulator (SIR) proteins to generate silent chromatin (60, 61). A high molecular weight complex isolated from *Drosophila* embryos was recently shown to contain HP1/ORC-associated protein (HOAP) in addition to HP1 and ORCs. HOAP has sequence similarity to high mobility group proteins and binds to satellite sequences *in vitro* (62). Mutations in the genes encoding ORC proteins and HOAP are suppressors of PEV, suggesting a role in heterochromatin formation (62).

What regulates HP1 association with protein partners? Post-translational modifications are likely to govern interactions between HP1 and partner proteins and/or itself. In *Drosophila*, HP1 is multiply phosphorylated giving rise to at least eight differently charged isoforms (63). *Drosophila* embryonic extracts possess HP1-containing complexes that differ in HP1 phosphorylation status (58, 62). For example, hypophosphorylated HP1 is found in a complex containing ORC and HOAP (58, 62). In *Drosophila*, casein kinase II (CKII) is credited for the phosphorylation of serine residues at the amino and carboxyl termini of HP1. Mutation of these serine residues to alanine reduces the amount of HP1 localized to centric heterochromatin and reduces gene silencing, suggesting phosphorylation plays a role in chromosome association and/or complex stability (63, 64).

In mammals, HP1 phosphorylation changes through the cell cycle. $HP1^{Hs\alpha}$ and $HP1^{Hs\gamma}$ exhibit increased levels of phosphorylation during mitosis (23). $HP1^{Hs\gamma}$ is a substrate for Pim-1 kinase that phosphorylates a serine cluster in the center of the protein (65). Phosphorylation of $HP1^{Hs\alpha}$ is thought to disrupt protein–protein interactions that are necessary to maintain most of the centric localization. In G2, phosphorylated $HP1^{Hs\alpha}$ shifts from a centric location to being dispersed throughout the nucleus. Clearly, the role of phosphorylation needs further investigation to understand the biological significance of this dynamic process.

Table 1. HP1 interacting partners and candidate partners

Protein	Organism	HP1 variant	Methodology	HP1 domain	Ref(s).
Transcription regulation/ chromatin modifying proteins					
H1	*Drosophila*	HP1	rPD	nd	46
HP1-BP74 H1-like	Mouse	mHP1α	Y2H, FW, rPD	Hinge region	46, 86
H3	Mouse	mHP1α, M31, M32	FW, rPD, exIP	CD	46, 54
H3	*Drosophila*	HP1	rPD	nd	46
Methylated K9 of H3	*S. pombe*	Swi6	rPD, ChIP	CD	38, 43
Methylated K9 of H3	*Drosophila*	HP1	IF, FAITC, NMR	CD	26, 41
Methylated K9 of H3	Mouse	mHP1α, M31, M32	rPD	CD	39
Methylated K9 of H3	Human	$HP1^{Hs\alpha}$, $HP1^{Hs\beta}$, $HP1^{Hs\gamma}$	rPD, SPRA	CD	38
H4	Mouse	M31	rPD	nd	54
H4	*Drosophila*	HP1	*In vitro* cross-linking	CSD	34
MacroH2A1.2*	Mouse	M31	IF	nd	87
SUVAR3–9	*Drosophila*	HP1	IF, Y2H, exIP	CSD	42
Suv39h1	Mouse	M31	IF, exIP, SED	nd	88, 89
SUV39H1	Human	$HP1^{Hs\beta}$	IF, exIP, SED	nd	88, 89
Suvar3–7	*Drosophila*	HP1	IF, Y2H, exIP	CSD	35, 50
KAP-1/TIF1β	Human	$HP1^{Hs\alpha}$, $HP1^{Hs\gamma}$	IF, rPD, exIP, SPRA, GFC	CSD	28, 46–48
KAP-1/TIF1β	Mouse	mHP1α, M31, M32	IF, rPD, Y2H, exIP, GFC	CSD	28, 37, 47, 86, 90
TRF1/PIN2	Mouse	M31	IF	nd	91
$TAF_{II}130$	Human	$HP1^{Hs\alpha}$, $HP1^{Hs\gamma}$	Y2H, transPD, exPD	CSD	36
TIF1α	Mouse	mHP1α, M31, M32	Y2H, rPD	CSD	86, 90
mSNF2β	Mouse	mHP1α	Y2H	CSD	86
Rb	Human	HP1, $HP1^{Hs\gamma}$	Y2H, exPD, exIP, ChIP	nd	57, 82
Rb	Maize	$HP1^{\gamma}$	rPD, Y2H	nd	82
Dnmt3a	Mouse cells	mHP1α	IF	nd	92
Dnmt3b	Mouse cells	mHP1α	IF	nd	92
ATRX/HP1-BP38	Mouse	mHP1α, M31	Y2H, IF	CSD	86, 93
Pim-1	Human	$HP1^{Hs\gamma}$	Y2H, exIP, rPD	CSD	65
CKII	*Drosophila*	HP1	*In vitro* phosphorylation	nd	63
dAF10	*Drosophila*	HP1	transPD	CSD	94
DNA replication and repair					
CAF-1 p150	Mouse	mHP1α, M31	IF, Y2H, rPD, GFC, NMR	CSD	28, 37
CAF-1 p150	Human	$HP1^{Hs\alpha}$	rPD	CSD	48
Ku70	Human	$HP1^{Hs\alpha}$	Y2H, rPD, exIP	CSD	95
BRCA-1*	Human	$HP1^{Hs\alpha}$	IF	nd	96
ORC1	*Drosophila*	HP1	transIP	CD, CSD	58
ORC2	*Drosophila*	HP1	IF, exPD, exIP, transIP	CD, CSD	58
ORC3	*Drosophila*	HP1	transIP	CD, CSD	58
ORC4	*Drosophila*	HP1	transIP	CD, CSD	58
ORC5	*Drosophila*	HP1	exIP, transIP	CD, CSD	58
ORC6	*Drosophila*	HP1	exIP, transIP	CD, CSD	58
Xorc1	*Xenopus*	$XHP1^{\alpha}$, $XHP1^{\gamma}$	Y2H	nd	58
HOAP	*Drosophila*	HP1	IF, exIP	nd	62
Nuclear architecture					
Lamin B receptor	Human	$HP1^{Hs\alpha}$, $HP1^{Hs\beta}$, $HP1^{Hs\gamma}$	Y2H, rPD, exPD, transPD, exIP	CSD	48, 52–54
HP1-BP84	Mouse	mHP1α, M31	Y2H	CSD	86
Lamin B	Mouse	M31	BA	CD	55
LAP2β	Mouse	M31	BA	CD	55
Nuclear envelope	Mouse	mHP1α, M31, M32	IF, BA	CD	55
Other chromosome-associated proteins					
Psc3	*S. pombe*	Swi6	IF, Y2H, exPD, ChIP	CD+Glu-rich	13
DDP1	*Drosophila*	HP1	IF	nd	97
Arp4/dArp6	*Drosophila*	HP1	IF	nd	98, 99
INCENP	Human	$HP1^{Hs\alpha}$, $HP1^{Hs\gamma}$	Y2H, transPD	Hinge region	100
Ki-67	Human	mHP1α, M31, M32	Y2H, exPD, IF	CSD	101
SP100B	Human	$HP1^{Hs\alpha}$, $HP1^{Hs\beta}$, $HP1^{Hs\gamma}$	IF, Y2H, rPD, transPD	CSD	48, 102, 103
EST AA153281	Mouse	mHP1α, M31	Y2H, rPD	CSD	37
EST AA003533	Mouse	mHP1α, M31	Y2H, rPD	CSD	37

BA, binding assay; ChIP, chromatin immunoprecipitation; exIP, co-immunoprecipitation using extract; exPD, pull-down assay using extracts; FAITC, fluorescence anisotropy, isothermal titration calorimetry; FW, far Western analysis; GFC, gel filtration chromatography; IF, immunofluorescence colocalization; nd, not determined; rIP, coprecipitation using recombinant proteins; rPD, pull-down assay using recombinant proteins; transIP, immunoprecipitation with *in vitro*-translated protein; transPD, pull-down assay using *in vitro* translated protein; SED, sedimentation assay; SPRA, surface plasmon resonance analysis; Y2H, yeast two-hybrid assay.
*Denotes cell cycle-dependent association.

HP1 Regulates Gene Expression

Effects on Gene Expression Near Centric Heterochromatin. The gene encoding HP1, *Su(var)2–5*, was isolated in a screen for suppressors and enhancers of PEV of the *white*$^+$ gene brought into juxtaposition with heterochromatin through a chromosomal rearrangement (8). To determine the effects of HP1 on chromatin packaging, stocks containing the well-characterized *Drosophila hsp26* gene inserted within centric heterochromatin were used for chromatin structure analysis (16). These transgenes exhibit less accessibility to nucleases and are packaged into a more regular nucleosome array than euchromatic insertions. In an HP1 mutant background the transgenes become more accessible to restriction enzyme digestion, indicating a more "open" chromatin configuration (66). To determine the transcriptional mechanism impaired by packaging with HP1, high-resolution chromatin structure analysis was performed (67). The results indicated that general transcription factors such as TFIID and RNA polymerase II are not associated with heterochromatic transgenes exhibiting silencing. Thus, association of HP1 correlates with a "closed" chromatin configuration that limits the accessibility of regulatory sites to trans-acting factors. This is similar to the mechanism of X-chromosome inactivation in mammals where transcription factors are also absent from genes on the inactive X (68), but contrasts the mechanism of silencing at the mating type loci in *S. cerevisiae* and Polycomb-mediated silencing in *Drosophila* in which general transcription factors and RNA polymerase II are found in association with silenced promoters (69–71). Differences between these systems and HP1 silencing could reflect fundamentally different properties of the silencing systems or developmentally different stages of silent chromatin maturation.

In contrast to the silencing effects HP1 has on euchromatic genes, HP1 is required for the expression of genes that naturally reside within heterochromatin. Over the years genetic and molecular analyses have revealed genes that reside within centric heterochromatin (72, 73). Two well-characterized genes are *light*, an essential gene encoding a protein involved in the vesicle transport pathway (74, 75), and *rolled*, an essential mitogen-activated protein kinase (76). Heterochromatic genes are not specific for *Drosophila*; they also have been discovered in *Arabidopsis* (77) and are likely to be found in other organisms as genomic sequence analysis becomes more complete. In *Drosophila*, heterochromatic genes appear to be unrelated to each other in function, however, they do share some common properties. Structurally, many heterochromatic genes have introns containing middle repetitive DNA sequences (74) (D. E. Cryderman and L.L.W., unpublished data). Heterochromatic genes are inefficiently expressed and sometimes exhibit PEV when translocated to euchromatin (78, 79). In addition, heterochromatic genes require heterochromatin proteins such as HP1 for expression (11).

How does HP1 establish a chromatin configuration that hinders the expression of euchromatic genes while fostering the expression of heterochromatic genes? This question will be better addressed as the promoter regions of heterochromatic genes are analyzed. Assuming a role for HP1 in chromatin compaction, HP1 might bring distant regulatory elements in association with the promoter region of heterochromatic genes. Alternatively, HP1 may be required to set up a favorable chromatin configuration within the promoter proximal region and/or be involved in the recruitment of general transcription factors as suggested by a recent report showing an interaction between HP1 and the general transcription factor $TF_{II}130$ (36).

Effects of Tethering HP1. What are the effects of HP1 on gene expression at locations other than centric heterochromatin? One approach taken to address this question has been to generate HP1 fusion proteins containing heterologous DNA binding domains. In mammalian cell transient transfection assays, effects of HP1 fusion proteins on the expression of reporter genes possessing the appropriate DNA binding sequences are assayed. In these experiments both murine and human HP1 family members repress transcription when tethered to a small number of sites located in close proximity to the promoter (36). Transcriptional repression no longer occurs as the binding sites are moved to distances more than 2 kb from the promoter (80). Does this indicate that HP1-mediated repression has only short-range capabilities, perhaps operating on a gene-by-gene basis? If this is the case, HP1 located at euchromatic sites (Fig. 1) might play a role in the regulation of individual euchromatic genes, rather than entire domains.

The effects of tethering HP1 in a chromosomal context, as in transgenic *Drosophila*, demonstrate the complexities of gene silencing. A Gal4–HP1 fusion protein tethered upstream of a reporter gene caused silencing at only one of six genomic locations tested (51, 81). Interestingly, the site that supported silencing was flanked by middle repetitive DNA sequences, reminiscent of heterochromatin domains. In this case, silencing could spread in trans to a homologue lacking the tethering sites. These results suggest that not all chromosomal contexts will support the formation of silent chromatin by HP1. The inability to silence at certain locations might depend on the chromatin of the neighboring region, including the types of histone modifications, as well as gene density and transcriptional status of the region.

Identification of HP1 Target Genes. As a second approach to determine the effects of HP1 on gene expression at locations other than centric heterochromatin, investigators have identified potential target genes by their response to HP1 dosage. Representational difference analysis identified two genes that are up-regulated in homozygous HP1 mutant larvae (59). Interestingly, one of the genes misregulated in the HP1 homozygous mutant maps to cytological region 31, a chromosome division that stains intensely with antibodies to HP1 (7). Two additional randomly selected genes within region 31 show up-regulation in HP1 homozygous mutants (59). For all four candidate HP1 target genes, mutations in additional modifiers of PEV, including *Su(var)3–9*, cause increases in gene expression. These results suggest that HP1 might function to silence genes located within euchromatic domains in a mechanism similar to that operating in centric regions.

A microarray approach has also been used to identify candidate genes regulated by HP1. Several hundred genes mapping within the euchromatic arms are up-regulated in an HP1 mutant background (D. E. Cryderman and L.L.W., unpublished data). In addition, several hundred genes were down-regulated, a pattern similar to that of heterochromatic genes. For all of the candidate target genes identified in *Drosophila* to date, it remains to be determined whether misregulation is caused by a direct interaction with HP1. It will be of interest to determine whether interactions between HP1 and specific genes are conserved through evolution.

In mammals, it is also likely that HP1 plays a role in the regulation of genes at noncentric locations. HP1 has been identified as a partner protein for many promoter-associated factors involved in control of gene repression. These include the transcription intermediate factor TIF1β that interacts with zinc finger proteins containing Krüppel-associated box (KRAB) domains known to be involved in transcriptional repression. The role of HP1 in KRAB-mediated repression is not clear, but might involve recruitment of histone deacetylases (47, 48).

The first example of a direct association of HP1 with a promoter region came from studies on the *cyclin E* gene (57). Binding of the Rb protein upstream of the *cyclin E* promoter

causes gene silencing, partly through the recruitment of histone deacetylases. In addition, chromatin cross-linking and immunoprecipitation experiments place Rb at the promoter with HP1 and methylated lysine nine of histone H3 (57). Consistent with the finding, an "Rb binding motif" is present within the amino acid sequences of HP1 from a variety of species (82). It is unclear whether HP1 is recruited to the *cyclin E* promoter by Rb, methylated lysine nine of histone H3, SUV39H1, or any combination of these interacting molecules. In support of such interactions, HP1 possesses the ability to simultaneously interact with the methylated histone H3 tail and Rb (57). One hypothesis is that Rb recruits histone deacetylases first, because the histone H3 methyltansferase cannot use an acetylated lysine as a substrate for methylation (43), then SUV39H1 methylates the histone tail which serves as the substrate for HP1 binding. Interestingly, *Drosophila* SU(VAR)3–9 was recently purified in a complex with histone deacetylase HDAC1, suggesting that the two proteins might cooperate to methylate previously acetylated histone tails (83).

The identification of HP1 target genes has implications for understanding breast cancer metastasis in humans. $HP1^{Hs\alpha}$, but not $HP1^{Hs\beta}$ or $HP1^{Hs\gamma}$, is down-regulated in highly invasive/metastatic breast cancer cells compared with poorly invasive/nonmetastatic breast cancer cells (84). Introduction of a tagged $HP1^{Hs\alpha}$ into the highly invasive/metastatic cells, which normally have low levels of $HP1^{Hs\alpha}$, lead to a less *in vitro* invasive phenotype. These results imply that modulation of the levels of $HP1^{Hs\alpha}$ alters molecular properties of cells needed for invasion. Consistent with the cell culture studies, $HP1^{Hs\alpha}$ is down-regulated in tissues from distant metastatic sites in breast cancer patients (84). One hypothesis is that $HP1^{Hs\alpha}$ normally silences genes required for metastasis, making $HP1^{Hs\alpha}$ a candidate metastasis suppressor. Depending on when $HP1^{Hs\alpha}$ is down-regulated during tumor progression, $HP1^{Hs\alpha}$ could be used as a predictive/prognostic marker for metastatic breast cancer.

Since the discovery of HP1 over 12 years ago by the laboratory of Sarah C. R. Elgin (Washington University, St. Louis), HP1 has grown in popularity. In part, this has been caused by the fact that HP1 has unexpectedly been identified as an interacting partner for a wide variety of proteins with diverse nuclear functions. Dissecting the function of HP1 in association with its partner proteins lies ahead. These experiments will shed light on the connections between chromatin structure, gene expression, DNA replication and repair, and nuclear organization.

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"Biology of Prostate Cancer"

Monday
October 14

POSTER
SESSION #2

5:00 pm – 7:00 pm

$HP1^{Hs\alpha}$ and breast cancer metastasis

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Heterochromatin protein 1 (HP1), a conserved chromosomal protein that localizes to heterochromatic regions of the genome, plays a role in silencing euchromatic genes brought into juxtaposition with heterochromatin by chromosome rearrangements or transposition events. A role for $HP1^{Hs\alpha}$, a human HP1 family member, in the regulation of genes involved in breast cancer invasion and metastasis has been suggested. This is based on the discovery that $HP1^{Hs\alpha}$ is down regulated in highly invasive/metastatic breast cancer cells compared to poorly invasive/non-metastatic breast cancer cells. Furthermore, expressing $HP1^{Hs\alpha}$ in highly invasive/metastatic breast cancer cell lines correlates with reducted invasion. To better understand the role of $HP1^{Hs\alpha}$ in breast cancer metastasis, we are examining the transcriptional regulation of the *$HP1^{Hs\alpha}$* gene. *$HP1^{Hs\alpha}$* promoter fragments were cloned into a luciferase reporter construct and transiently tranfected into both a highly invasive/metastatic breast cancer cell line and a poorly invasive/non-metastatic breast cancer cell line. Luciferase activity is being measured to identify regulatory elements responsible for *$HP1^{Hs\alpha}$* regulation. The results will be used to identify the *cis*-regulatory elements responsible for differential regulation of *$HP1^{Hs\alpha}$* in poorly invasive/non-metastatic and highly invasive/metastatic breast cancer cells.
To determine whether $HP1^{Hs\alpha}$, which has 44% amino acid sequence identity to *Drosophila* HP1, is a functional homolog of the *Drosophila* HP1 protein, we generated transgenic flies carrying the human *$HP1^{Hs\alpha}$* gene under control of the *hsp70* promoter. $HP1^{Hs\alpha}$ localizes to heterochromatic regions on polytene chromosomes in a similar pattern as *Drosophila* HP1. Overexpression of Drosophila HP1 has been shown to enhance silencing of transgenes within centric heterochromatin. Overexpression of *$HP1^{Hs\alpha}$* also enhances silencing of these same transgenes. Finally, *$HP1^{Hs\alpha}$* rescues the lethal phenotype associated with HP1 mutantions. Collectively, these results suggest that $HP1^{Hs\alpha}$ is a functional homolog of *Drosophila* HP1.