RESEARCH PAPER

Transcription 2:2, 95-99; March/April 2011; © 2011 Landes Bioscience

Heterochromatin protein 1a is required for an open chromatin structure

Diane E. Cryderman, Michael W. Vitalini and Lori L. Wallrath*

Department of Biochemistry; University of Iowa; Iowa City, IA USA

Key words: chromatin, Drosophila, gene regulation, heterochromatin, Heterochromatin Protein 1, histone methyltransferase

The *Drosophila melanogaster* fourth chromosome contains interspersed domains of active and repressive chromatin. We investigated a stock harboring a silenced transgene inserted into *Dyrk3* and near *Caps*—two expressed genes on chromosome four. In an HP1a-deficient background, transgene expression was activated while, paradoxically, expression of *Dyrk3* and *Caps* was reduced. We found that the promoters of *Dyrk3* and *Caps* contained DNase I hypersensitive sites but also possessed methylated histone H3 and HP1a, marks of repressive chromatin. In HP1a-deficient flies, the *Dyrk3* and *Caps* promoters displayed diminished accessibility to nuclease digestion, revealing a surprising role for HP1a in opening chromatin.

Eukaryotic genomes contain characteristic patterns of active and repressive chromatin, each harboring distinctly modified histones and associated factors.[1] Histone N-terminal tails are methylated on various residues, which triggers the recruitment of partner proteins that affect chromatin structure. For example, centric heterochromatin, a form of repressive chromatin, is enriched for histone H3 that is di- and tri-methylated at lysine 9 (H3K9me2/3).[2] H3K9me2/3 is a binding site for other factors associated with gene silencing, such as Heterochromatin Protein 1a (HP1a). Histone H3 can also be methylated on lysine 4 (H3K4me), a modification enriched in euchromatin. The H3K4me mark binds chromatin remodeling complexes (e.g., NURF) that activate gene expression.[3]

In Drosophila, when euchromatic genes are brought into juxtaposition with heterochromatin, they are silenced in a mosaic pattern; this phenomenon, known as position-effect variegation (PEV), implies that heterochromatin spreads into transposed euchromatin.[4] PEV can be suppressed or enhanced by second-site mutations termed *Su(var)s* and *E(var)s*, respectively,[4] which implies these mutations disrupt the formation and/or spread of heterochromatin.

Two *Su(var)* genes, *Su(var)2-5* and *Su(var)3-9*, are of particular interest because they are part of a positive feedback loop predicted to propagate and maintain heterochromatin. *Su(var)3-9* encodes a histone methyltransferase (HMT) that generates H3K9me.[5,6] In turn, H3K9me2/3 is a binding site for HP1a [the protein product of *Su(var)2-5*]. HP1a is a conserved chromo domain protein[7,8] that localizes to centric heterochromatin. HP1a is part of a protein family in which members posses both a chromodomain and chromoshadow domain, yet have diverse functions, including sex-specific roles.[9] As loss of HP1a is lethal, none of the family members appear to have redundant function with HP1a. Within heterochromatin, HP1a is thought to recruit SU(VAR)3-9, which methylates adjacent histone H3 tails, generating additional HP1a binding sites and completing the positive feed-back loop.

Although euchromatic genes brought near heterochromatin are silenced, silencing can be relieved by *Su(var)* mutations and chromosomal translocations that return the gene to euchromatin.[4] Conversely, genes that naturally reside in heterochromatin are not expressed well in *Su(var)* mutant backgrounds, or when placed near euchromatin by chromosomal translocations.[10-13] The underlying cause of this reciprocal expression pattern is elusive; however, it may reflect an unexpected role for HP1a in gene expression.

The small fourth chromosome of Drosophila provides an attractive environment to study the role of HP1a in gene regulation because it uniquely contains interspersed domains of active and repressive chromatin.[14] As with other Drosophila chromosomes, repressive domains on chromosome four are associated with H3K9me2/3; but, unique to chromosome four, this H3K9me2/3 is primarily generated by a different HMT—dSETDB1.[15,16] While repressive domains on the fourth chromosome share some characteristics of centric heterochromatin, they differ in several respects.[14] It is important to note that repressive domains can express resident genes. To take advantage of the unique situation of active and repressive domains, we examined the expression of a variegating *hsp70-white* P-element inserted in the fourth chromosome within the coding region of the expressed gene *Dyrk3*. The position of this insert provided an opportunity to better understand the mechanisms underlying differential control of gene expression by HP1a.

Here, we present data consistent with gene-specific roles for HP1a. This unique transgene insertion site revealed that

*Correspondence to: Lori L. Wallrath; Email: lori-wallrath@uiowa.edu
Submitted: 11/03/10; Revised: 12/30/10; Accepted: 12/31/10
DOI: 10.4161/trns.2.2.14687

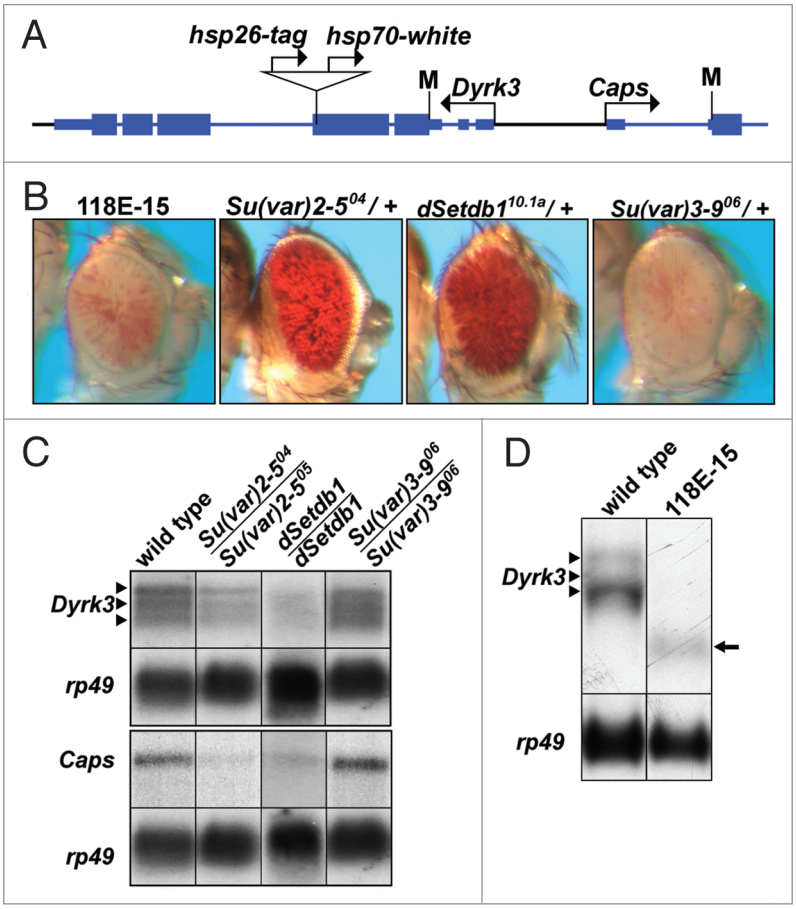


Figure 1. An *hsp70-white* transgene inserted within *Dyrk3* displays variegating expression. (A) Schematic representation of *Dyrk3* and a portion of *Caps* (modified from the RefSeq track of the UCSC Genome Browser). Bent arrows represent the transcription start sites (+1). Coding sequences are displayed as thick, blue boxes; transcribed, but not translated sequences are shown as thin, blue boxes and introns shown as thin, blue lines. The translation start sites (M) and the location of the P-element insertion (triangle) are indicated. (B) Eye phenotypes of strains containing the *hsp70-white* transgene. Females of the P-element insertion stock, 118E-15, were crossed to males containing mutations in known modifiers of PEV. Photographs of eyes from the parental stock and the resulting heterozygous progeny are shown. (C) Northern analysis of *Dyrk3* and *Caps*. RNA was isolated from third instar larvae of y,w^{67c23} (wild type) and *Su(var)2-5*, *dSetdb1* or *Su(var)3-9* mutants. Transcripts were detected by hybridization with an α-^{32}P labeled fragment for *Dyrk3* or *Caps*. Detection of *rp49* was used for loading control. Three arrowheads indicate alternatively spliced *Dyrk3* transcripts. Results shown are representative of three experiments. (D) Northern analysis of *Dyrk3* in a wild-type background and stock 118E-15. Arrow heads indicate wild-type transcripts, arrow indicates the truncated transcripts observed in the P-element-containing 118E-15 stock.

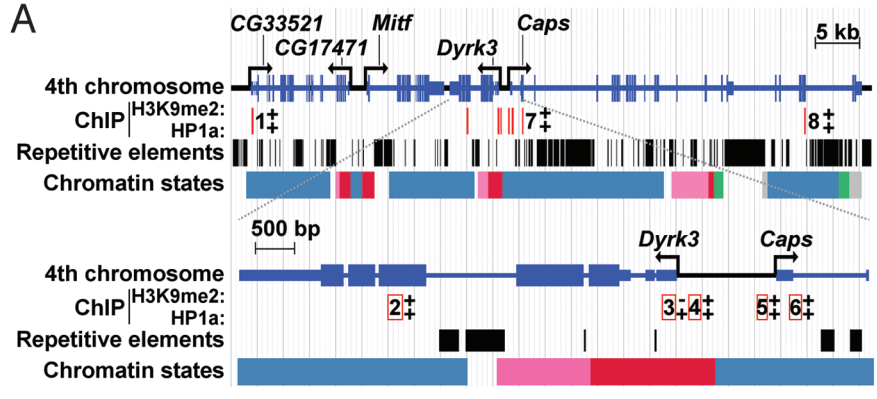
HP1a-dependent gene expression and HP1a-dependent gene silencing can occur at nearly the same genomic location. Surprisingly, HP1a was required to establish or maintain an open chromatin structure at the promoter of *Dyrk3*, while, at the same time repressing expression of the *hsp70-white* reporter. These results suggest HP1a supports gene expression via a previously undiscovered mechanism that involves the generation of open chromatin.

Previously we isolated stock 118E-15,[17,18] in which an *hsp70-white* reporter gene inserted within the coding region of *Dyrk3* (which encodes a serine/threonine kinase; **Fig. 1A**). PEV of the reporter was suppressed by a null allele of *Su(var)2-5,* demonstrating that the variegated silencing required HP1a (**Fig. 1B**).[18] Mutations in the histone H3K9 HMT-encoding *dSetdb1* also suppressed silencing of the reporter gene, whereas alleles of *Su(var)3-9* did not, consistent with dSETDB1 functioning as the primary methyltransferase acting on the fourth chromosome.[15,16,19]

PEV of the *hsp70-white* reporter gene suggested that the P-element had inserted into a region of repressive chromatin. Surprisingly, northern analyses revealed that *Dyrk3* and *Caps* (a neighboring gene located ~1.2 kb upstream of *Dyrk3*; **Fig. 1C**) were expressed. The P-element insertion did not prevent transcription initiation from the *Dyrk3* promoter as evidenced by a shorter length message, consistent with transcriptional termination within the P-element (**Fig. 1D**). In larvae trans-heterozygous for null *Su(var)2-5* or *dSetdb1* alleles, expression levels of *Dyrk3* and *Caps* were reduced by 50% or more (**Fig. 1C**). In contrast, a stock homozygous for a null allele of *Su(var)3-9* expressed both transcripts similar to wild type. Despite a recently observed connection between HP1a and splicing factors,[20] the mutant background did not appear to alter splicing of *Dyrk3*. Taken together, these results suggest that *Dyrk3* and *Caps* reside in a repressive chromatin environment that requires dSETDB1 and HP1a for full transcriptional activity.

The region surrounding *Dyrk3* and *Caps* is characteristic of much of the fourth chromosome: it is enriched for repetitive elements, but maintains a gene density similar to that of euchromatic regions (**Fig. 2**).[14] The requirement of HP1a for two apparently opposite effects—active transcription of *Dyrk3* and *Caps*, and PEV silencing of the reporter gene—led us to test whether HP1a is directly associated with the chromatin of this region. Approximately 70 kb of chromosome four, centered around *Dyrk3* and *Caps* were analyzed via chromatin immunoprecipitation (ChIP). Third instar salivary gland nuclei were used for ChIP as available antibodies recognized multiple proteins in other tissues upon western analysis.[21,22] Expression of *Dyrk3* and *Caps* was readily detected by RT-PCR using RNA isolated from salivary glands and showed a 7- to 11-fold decrease in expression in the *Su(var)2-5* mutant background (data not shown). Genomic fragments were immunoprecipitated using either anti-H3K9me2 or anti-HP1a antibodies, and amplified using eight unique primer sets (**Fig. 2A and B**). Antibodies recognizing H3K9me2 retrieved genomic fragments amplified by seven of eight primer sets, with a signal ranging from 1.89–3.60% of input (compared to 0.5% for the negative control; **Fig. 2B**). The exception was a region adjacent to the *Dyrk3* transcription start site (TSS; +23 to +183), which gave values similar to the negative control (**Fig. 2B**). Surprisingly, all primer sets demonstrated that HP1a

was associated with the target sequences; this included the primer set that was negative for histone H3K9me2. Values ranged from 1.33–3.07% of input (compared to 0.66% for the negative control; **Fig. 2B**). Data from the modENCODE project agreed with our findings, showing that *Dyrk3* and *Caps* are expressed and are associated with HP1a and H3K9me2/3 in S2 cultured cells and during development, including the third instar larval stage (**Fig. 2A**; www.modencode.org). Histone H3K27me, a mark of developmental silencing via Polycomb was depleted throughout the region. modENCODE has annotated chromatin to include nine distinctive states.[23] The TSS of *Dyrk3* was designated as state 1, characterized by H3K4me2/3 in combination with H3K9ac; both are marks of active chromatin. The coding regions of both *Dyrk3* and *Caps* possess regions in state 2, which are enriched in H3K36me3, a signature of transcription elongation. The entire genomic region, including some exons of *Dyrk3* and *Caps* are designated as state 7, characterized by high levels of H3K9me2/3 similar to that present in centric heterochromatin and other regions of chromosome 4,[23] (**Fig. 2A**). Taken together, these results suggest that HP1a associates with the genomic region encompassing *Dyrk3* and *Caps*, even at sites where levels of histone H3K9me2 are undetectable and that HP1a promotes expression of *Dyrk3* and *Caps*.



B

Primer Set	% input HP1a	p	% input H3K9me2	p
1	1.53 +/- 0.0	0.01	2.79 +/- 0.57	0.02
2	2.55 +/- 0.0	0.05	3.57 +/- 0.82	0.01
3	1.41 +/- 0.0	0.00	0.53 +/- 0.13	0.45
4	3.07 +/- 0.0	0.00	1.89 +/- 0.11	0.00
5	2.20 +/- 0.5	0.03	2.81 +/- 0.77	0.06
6	2.00 +/- 0.3	0.01	3.60 +/- 0.45	0.00
7	1.33 +/- 0.0	0.00	3.10 +/- 0.57	0.02
8	2.38 +/- 0.0	0.02	3.50 +/- 0.65	0.01
+ control	2.77 +/- 0.0	0.03	11.20 +/- 0.02	0.00
- control	0.66 +/- 0.0	0.57	0.50 +/- 0.50	0.77

Figure 2. The distribution of HP1a, histone H3K9me2, repetitive elements and chromatin states within the *Dyrk3/Caps* (A) genomic region. A diagram of the sub-telomeric region of the fourth chromosome is shown at the top; a magnification of *Dyrk3* and the 5' region of *Caps* is depicted below. Repetitive elements are represented by black boxes (taken from Repeat Masker track of the UCSD Genome Browser). Polyclonal antibodies to HP1a (Covance), H3K9me2 (Upstate) and GFP (Molecular probes) were used for ChIP from third instar salivary glands. The location of primer sets are indicated by red lines; numbers adjacent to (top) or within the open red boxes (bottom) correspond to the number of a primer set. +, a positive ChIP signal; -, a negative result. Primers for *CG31999* at position 102B1 on chromosome 4 were used as a negative control. Primers corresponding to an *hsp26* transgene integrated near the centric region of chromosome 4 were used as a positive control. This transgene is silenced in an HP1a-dependent manner.[18] The chromatin states are described according to modENCODE:[23] red represents enrichment of H3K4me3/2 and H3K9Ac (state 1) found at TSSs, pink represents enrichment for H3K36me3 (state 2) associated with transcription elongation; green represents enrichment for H4K16ac(state 5); blue represents enrichment for H3K9me2/3, which is found in heterochromatin (state 7); grey represents regions of very low transcriptional activity (state 9). (B) Data obtained from ChIP experiments; primer set numbers correspond to numbers in (A) and p denotes p values.

HP1a and H3K9me2/3 associate with sequences throughout the 70 kb encompassing *Dyrk3* and *Caps*, suggesting that this region might be assembled into closed chromatin, a structure suggested by the PEV of the *hsp70-white* transgene.[18] Paradoxically, this region actively expresses both *Dyrk3* and *Caps*, suggesting it is in an open conformation. To explore this paradox, we used DNase I hypersensitivity assays to determine the chromatin conformation of the intergenic region between the *Dyrk3* and the *Caps* TSS (**Fig. 3**). Consistent with active transcription, both the wild-type host stock and the P-element-containing 118E-15 stock showed three prominent DNase I hypersensitive sites, which corresponded approximately to positions +460, +160 and -240, relative to the *Dyrk3* TSS (**Fig. 3**). Additionally, we detected a fourth hypersensitive site, at approximately position -130 relative to the *Caps* transcription start site (**Fig. 3**). Collectively, these data demonstrate that although HP1a associates with this region, the chromatin assumes an open conformation near the promoters of these two genes; in addition, insertion of the P-element had no effect on the open chromatin conformation of the endogenous genes.

In a *Su(var)2-5* mutant background, *Dyrk3* and *Caps* are downregulated. This suggests that, in the absence of HP1a, these genes assume a chromatin structure that is less transcriptionally active. To test this unexpected hypothesis, we performed restriction enzyme accessibility assays, which allow quantitative measurements of chromatin accessibility.[17,18] In performing this assay, we took advantage of several *Ava*II restriction sites in the *Dyrk3* promoter region. One site lies 110 bp upstream of the *Dyrk3* transcription start site (approximately 270 bp and 130 bp from the DNase hypersensitive sites at +160 and -240, respectively); two more distal *Ava*II sites lie at -231 and -3,355 bp (**Fig. 4**). Nuclei from the wild-type host stock or *Su(var)2-5* trans-heterozygotes were treated with an excess of *Ava*II, processed, and the cleavage products were resolved by electrophoresis and visualized by a radiolabeled probe (**Fig. 4**). In nuclei from the wild-type stocks, 61–65% of the promoter-containing

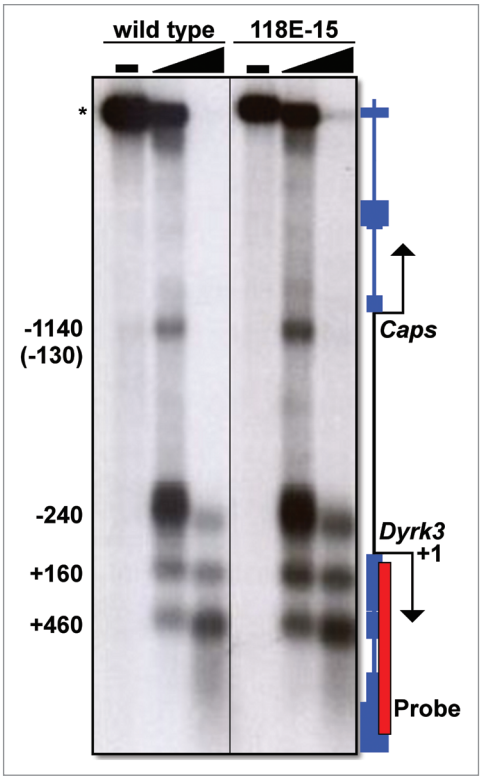


Figure 3. DNase I hypersensitivity analysis of the *Dyrk3* and *Caps* promoter regions. Nuclei were isolated from larvae and treated with increasing amounts of DNase I. Genomic DNA was purified and digested to completion with *Nci*I, which cleaves at sites flanking the region of interest. The DNA was separated by electrophoresis and transferred to a nylon membrane (Amersham). DNase I hypersensitive sites were detected following hybridization with α-^{32}P labeled 842 bp fragment of *Dyrk3* (red box). Numbers to the left of the radiograph denote the approximate locations of DNase I hypersensitive sites relative to the *Dyrk3* transcription start site (+1).The * denotes the NciI Fragment that is not cleaved by DNase 1. The number in parentheses represents the distance relative to the TSS of Caps.

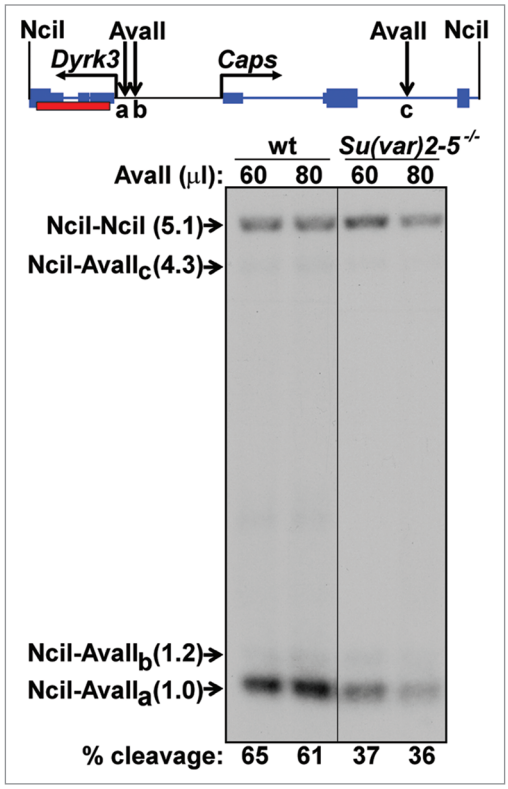


Figure 4. Restriction enzyme accessibility analysis of the *Dyrk3* and *Caps* promoter regions. Top: schematic of the *Dyrk3-Caps* region flanked by two *Nci*I sites, labels are the same as in **Figure 3**, with the addition of *Ava*II sites (down arrows). Bottom: Southern analysis of fragments resulting from nuclei isolated from wild type (wt) or trans-heterozygous [*Su(var)2-5*$^{-/-}$] larvae treated with *Ava*II. Purified genomic DNA was cut with NciI. The arrow indicates the position of the *Nci*I-*Nci*I parental fragment. The positions of the two fragments resulting from cleavage at the distal *Ava*II sites (B and C) are indicated. *Nci*I-*Ava*IIa indicates the position of the quantifiable fragment resulting from cleavage at the proximal *Ava*IIa site. The red box indicates a fragment of *Dyrk3* used as a probe. The percent cleavage at the proximal *Ava*II site is reported at the bottom. Cleavage at the *Ava*IIa site was quantified using Labworks software (UVP BioImaging Systems) and is reported as the percentage of the total signal from all four bands.

fragment was cleaved at the proximal *Ava*II site (**Fig.** 4), confirming the promoter assembles into an open chromatin conformation.[17,18] In a *Su(var)2-5* trans-heterozygous null background, the same assay detected this site to be cleaved with only 36–37% efficiency (**Fig.** 4), showing that in the absence of HP1a this promoter region tends to adopt a relatively closed conformation. Surprisingly, these data indicate that HP1a allows the *Dyrk3* promoter region to assume an open, transcriptionally active conformation.

Although heterochromatin contains proteins known to inhibit transcription from euchromatic genes, many transcriptionally competent genes are, in fact, embedded in heterochromatin.[24] Here, we provide insights into a mechanism that might drive this transcription by showing that the promoter of a gene normally residing in a repressive chromatin environment is held in an open conformation only if it is packaged by chromatin containing HP1a. Moreover, across the length of the 70 kb of chromosome four containing *Dyrk3* and *Caps*, we found HP1a associated with histone H3K9me2. These findings are consistent with recent reports that the epigenetic mark of histone H3K9me2 can be found associated with transcribed genes.[21,25,26] Thus, the association of HP1a did not necessarily silence transcription, providing an explanation for the transcription of genes that naturally reside in heterochromatin.[24] Most surprising, the *Dyrk3* promoter requires bound HP1a to display an open chromatin configuration.

Several models have been put forth to describe the expression of heterochromatic genes.[13] In *D. melanogaster*, studies comparing genes that reside in heterochromatin to their euchromatic orthologs in other Drosophila species found that the promoter structures are generally conserved.[27] This strongly suggests unique promoter elements do not drive the contrasting expression patterns. Thus, in heterochromatin, distant enhancer elements might drive differential expression from otherwise unremarkable promoters. Although repetitive elements frequently interrupt

heterochromatic genes and might displace enhancers, bound HP1a is predicted to dimerize at non-adjacent nucleosomes, allowing chromatin to loop, bringing distal enhancers in proximity with promoter elements.[28] Such a model would explain why heterochromatic genes require HP1.

HP1a supports expression of selective euchromatic genes, including genes within heat-shock and developmentally induced puffs.[20,21,29] In fact, HP1a interacts with, and stabilizes, transcripts from ~100 highly transcribed genes.[29] Recent studies into this effect uncovered that HP1a interacts with several heterogeneous nuclear ribonucleoproteins (hnRNPs).[20] HP1 proteins have also recently been connected with transcription elongation; however, the major player seems to be one of the other family members, HP1c.[30]

Our data suggest an additional mechanism by which HP1a supports gene expression. We observed that the transgene inserted into *Dyrk3* exhibited PEV, consistent with HP1a-dependent repressive chromatin in this region. Nevertheless, the open chromatin structure at the *Dyrk3* and *Caps* promoter regions requires HP1a, suggesting that, at these promoters, HP1a promotes an open chromatin structure. One possible explanation of our data may be that HP1a bound near the *Dyrk3* promoter recruits a chromatin-remodeling complex that locally opens the chromatin. Evidence for this comes from experiments in which overexpression of SNF2-type chromatin remodelers altered heterochromatic gene silencing.[31,32]

Acknowledgements

We would like to thank members of the Wallrath lab for comments on the manuscript, S. Mackey for assistance with photography, J. Danzer and J. Limas for technical assistance. This research was supported by a Ruth L. Kirschstein NRSA Postdoctoral Fellowship (GM085974) to M.W.V. and NIH Grant (GM61513) to L.L.W.

Authors' Contributions

D.E.C. performed gene expression/chromatin structure analysis and analyzed data; M.W.V. performed data analysis and prepared figures; L.L.W. designed experiments, analyzed the data and prepared the manuscript.

References

1. Mellor J, Dudek P, Clynes D. A glimpse into the epigenetic landscape of gene regulation. Curr Opin Genet Dev 2008; 18:116-22.
2. Kouzarides T. Chromatin modifications and their function. Cell 2007; 128:693-705.
3. Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, et al. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. Nature 2006; 442:86-90.
4. Weiler KS, Wakimoto BT. Heterochromatin and gene expression in Drosophila. Annu Rev Genet 1995; 29:577-605.
5. Eskeland R, Czermin B, Boeke J, Bonaldi T, Regula JT, Imhof A. The N-terminus of Drosophila SU(VAR)3-9 mediates dimerization and regulates its methyltransferase activity. Biochemistry 2004; 43:3740-9.
6. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 2001; 410:116-20.
7. Eissenberg JC, James TC, Foster-Hartnett DM, Hartnett T, Ngan V, Elgin SC. Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*. Proc Natl Acad Sci USA 1990; 87:9923-7.
8. Elgin SC, Grewal SI. Heterochromatin: silence is golden. Curr Biol 2003; 13:895-8.
9. Vermaak D, Henikoff S, Malik HS. Positive selection drives the evolution of rhino, a member of the heterochromatin protein 1 family in Drosophila. PLoS Genet 2005; 1:96-108.
10. Lu BY, Emtage PC, Duyf BJ, Hilliker AJ, Eissenberg JC. Heterochromatin protein 1 is required for the normal expression of two heterochromatin genes in Drosophila. Genetics 2000; 155:699-708.
11. Schulze SR, Sinclair DA, Fitzpatrick KA, Honda BM. A genetic and molecular characterization of two proximal heterochromatic genes on chromosome 3 of *Drosophila melanogaster*. Genetics 2005; 169:2165-77.
12. Wakimoto BT, Hearn MG. The effects of chromosome rearrangements on the expression of heterochromatic genes in chromosome 2L of *Drosophila melanogaster*. Genetics 1990; 125:141-54.
13. Yasuhara JC, Wakimoto BT. Oxymoron no more: the expanding world of heterochromatic genes. Trends Genet 2006; 22:330-8.
14. Riddle NC, Shaffer CD, Elgin SC. A lot about a little dot—lessons learned from *Drosophila melanogaster* chromosome 4. Biochem Cell Biol 2009; 87:229-41.
15. Seum C, Reo E, Peng H, Rauscher FJ, 3rd, Spierer P, Bontron S. Drosophila SETDB1 is required for chromosome 4 silencing. PLoS Genet 2007; 3:76.
16. Tzeng TY, Lee CH, Chan LW, Shen CK. Epigenetic regulation of the Drosophila chromosome 4 by the histone H3K9 methyltransferase dSETDB1. Proc Natl Acad Sci USA 2007; 104:12691-6.
17. Cryderman DE, Cuaycong MH, Elgin SC, Wallrath LL. Characterization of sequences associated with position-effect variegation at pericentric sites in Drosophila heterochromatin. Chromosoma 1998; 107:277-85.
18. Wallrath LL, Elgin SC. Position effect variegation in Drosophila is associated with an altered chromatin structure. Genes Dev 1995; 9:1263-77.
19. Brower-Toland B, Riddle NC, Jiang H, Huisinga KL, Elgin SC. Multiple SET methyltransferases are required to maintain normal heterochromatin domains in the genome of *Drosophila melanogaster*. Genetics 2009; 181:1303-19.
20. Piacentini L, Fanti L, Negri R, Del Vescovo V, Fatica A, Altieri F, et al. Heterochromatin protein 1 (HP1a) positively regulates euchromatic gene expression through RNA transcript association and interaction with hnRNPs in Drosophila. PLoS Genet 2009; 5:1000670.
21. Cryderman DE, Grade SK, Li Y, Fanti L, Pimpinelli S, Wallrath LL. Role of Drosophila HP1 in euchromatic gene expression. Dev Dyn 2005; 232:767-74.
22. Danzer JR, Wallrath LL. Mechanisms of HP1-mediated gene silencing in Drosophila. Development 2004; 131:3571-80.
23. Kharchenko PV, Alekseyenko AA, Schwartz YB. Comprehensive analysis of the chromatin land scape in *Drosophila melanogaster*. Nature 2010; In press.
24. Dimitri P, Caizzi R, Giordano E, Carmela Accardo M, Lattanzi G, Biamonti G. Constitutive heterochromatin: a surprising variety of expressed sequences. Chromosoma 2009; 118:419-35.
25. de Wit E, Greil F, van Steensel B. High-resolution mapping reveals links of HP1 with active and inactive chromatin components. PLoS Genet 2007; 3:38.
26. Yasuhara JC, Wakimoto BT. Molecular landscape of modified histones in Drosophila heterochromatic genes and euchromatin-heterochromatin transition zones. PLoS Genet 2008; 4:16.
27. Yasuhara JC, DeCrease CH, Wakimoto BT. Evolution of heterochromatic genes of Drosophila. Proc Natl Acad Sci USA 2005; 102:10958-63.
28. Li Y, Danzer JR, Alvarez P, Belmont AS, Wallrath LL. Effects of tethering HP1 to euchromatic regions of the Drosophila genome. Development 2003; 130:1817-24.
29. Piacentini L, Fanti L, Berloco M, Perrini B, Pimpinelli S. Heterochromatin protein 1 (HP1) is associated with induced gene expression in Drosophila euchromatin. J Cell Biol 2003; 161:707-14.
30. Kwon SH, Florens L, Swanson SK, Washburn MP, Abmayr SM, Workman JL. Heterochromatin protein 1 (HP1) connects the FACT histone chaperone complex to the phosphorylated CTD of RNA polymerase II. Genes Dev 2010; 24:2133-45.
31. Schneiderman JI, Sakai A, Goldstein S, Ahmad K. The XNP remodeler targets dynamic chromatin in Drosophila. Proc Natl Acad Sci USA 2009; 106:14472-7.
32. Sugiyama T, Cam HP, Sugiyama R, Noma K, Zofall M, Kobayashi R, et al. SHREC, an effector complex for heterochromatic transcriptional silencing. Cell 2007; 128:491-504.