Chromatin-Associated Proteins HP1 and Mod(mdg4) Modify Y-Linked Regulatory Variation in the *Drosophila* Testis

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ABSTRACT Chromatin remodeling is crucial for gene regulation. Remodeling is often mediated through chemical modifications of the DNA template, DNA-associated proteins, and RNA-mediated processes. Y-linked regulatory variation (YRV) refers to the quantitative effects that polymorphic tracts of Y-linked chromatin exert on gene expression of X-linked and autosomal genes. Here we show that naturally occurring polymorphisms in the *Drosophila melanogaster* Y chromosome contribute disproportionately to gene expression variation in the testis. The variation is dependent on wild-type expression levels of *mod(mdg4)* as well as *Su(var)205*; the latter gene codes for heterochromatin protein 1 (HP1) in *Drosophila*. Testis-specific YRV is abolished in genotypes with heterozygous loss-of-function mutations for *mod(mdg4)* and *Su(var)205* but not in similar experiments with *JIL-1*. Furthermore, the Y chromosome differentially regulates several ubiquitously expressed genes. The results highlight the requirement for wild-type dosage of *Su(var)205* and *mod(mdg4)* in enabling naturally occurring Y-linked regulatory variation in the testis. The phenotypes that emerge in the context of wild-type levels of the HP1 and Mod(mdg4) proteins might be part of an adaptive response to the environment.

CHRMATIN-REMODELING proteins are crucial in gene regulation and usually exert their function through chemical modifications of the DNA template, DNA-associated proteins, and RNA-mediated processes (Muller and Leutz 2001; Ng and Gurdon 2008; Brien and Bracken 2009). However, in fruit flies, DNA methylation is either absent or occurs at extremely low levels (Lyko *et al.* 2000; Phalke *et al.* 2009; Gou *et al.* 2010; Schaefer and Lyko 2010), such that the structure of chromatin might be entirely determined by protein modifications and RNA-mediated processes. The post-translational modifications of histones and DNA-binding proteins modulate the physical structure of DNA and ultimately regulate gene expression (Spencer and Davie 1999; Kouzarides 2007; Bonasio *et al.* 2010). Classical chromatin-remodeling proteins were classified based on their ability to

modulate the spreading of heterochromatin at the boundaries between the domains of euchromatin and heterochromatin. Accordingly, genetic screens identified chromatin regulators in *Drosophila* using the *white* gene allele *w(m4)*, in which the wild-type *white* gene is repositioned at a boundary between chromatin domains (position effect variegation, or PEV) (Ashburner *et al.* 2005). The studies uncovered >200 proteins with the ability to modulate chromatin states and enhance or suppress PEV (Henikoff 1990; Gelbart *et al.* 1997; Schotta *et al.* 2003; Ashburner *et al.* 2005; Tweedie *et al.* 2009). A small number of those modifiers have been mapped and functionally studied at the molecular level.

Heterochromatin protein 1 (HP1) is a classical non-histone chromatin protein. HP1 is encoded by the *Su(var)205* gene in *Drosophila* and localizes throughout the genome in euchromatic and heterochromatic sites. However, the protein is more abundant in segments of heterochromatin (de Wit *et al.* 2007; Yin *et al.* 2011). Recruitment of HP1 to chromatin is mediated by tri- or di-methylation of lysine 9 of histone 3 (H3K9m3 or H3K9me2), a chromatin mark established by the *Su(var)3-9* protein (Grewal and Moazed 2003; Grewal and Jia 2007). The ability of HP1 knockdown to rescue silencing of the *w(m4h)* gene marker shows that

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HP1 is a key factor in chromatin structure and a strong suppressor of variegation (red-eye phenotype). Other modifiers of chromatin include Mod(*mdg4*) and JIL-1. Mod(*mdg4*) is a classical modifier of variegation. The locus is complex, with evidence of *trans*-splicing during messenger RNA (mRNA) processing (Cai and Levine 1997; Buchner *et al.* 2000; Mongelard *et al.* 2002). Experimental evidence indicates that Mod(*mdg4*) is involved in meiosis I and chromosome segregation in males (Soltani-Bejnood *et al.* 2007), in the regulation of chromatin assembly or disassembly (Gerasimova *et al.* 1995), oogenesis (Buchner *et al.* 2000), and germ-cell migration (Read *et al.* 2000). JIL-1 has histone kinase activity (Jin *et al.* 1999; Rath *et al.* 2006; Zhang *et al.* 2006) and is involved in the negative regulation of chromatin silencing and in chromosome organization. Deficiency of JIL-1 leads to strong enhancer of variegation (white-eye phenotype), counteracting even the suppressor-of-variegation effect of HP1 and Su(*var*)3-9 (Wang *et al.* 2011).

Y-linked regulatory variation (YRV) refers to the quantitative effects that polymorphic tracts of Y-linked chromatin exert on gene regulation of X-linked and autosomal genes (Lemos *et al.* 2008). One hypothesis is that copy-number polymorphism in tracts of sequence repeats underlies cryptic regulatory variation emerging from Y chromosomes (Lemos *et al.* 2008). This expectation rests on the lack of polymorphic variation in Y-linked protein-coding genes as well as the finding that Y-linked repeats are highly variable (Lyckegaard and Clark 1989; Zurovcova and Eanes 1999; Paredes *et al.* 2011; Larracuente and Clark 2013). The observation that YRV occurs in XXY female genotypes, in which Y-linked protein-coding genes are not expressed, gives further strength to the notion that cryptic Y-linked repeats might cause YRV (Lemos *et al.* 2010). Finally, repeat variation in the Y-linked ribosomal DNA (rDNA) locus is consequential to the specification of chromatin states and genome-wide gene expression in *Drosophila* (Paredes and Maggert 2009; Paredes *et al.* 2011).

Here we addressed whether the expression of YRV is dependent on Su(*var*)205 dosage. In addition, we tested the chromatin remodelers mod(*mdg4*) and JIL-1 for their ability to modify YRV in a Y-chromosome-dependent manner. We found that, independently of Y-chromosome background, Su(*var*)205 and mod(*mdg4*) strongly suppress PEV, whereas JIL-1 strongly enhances PEV. Furthermore, we addressed the contribution of these genes to the manifestation of testis-specific and somatic YRV. Specifically, we found that *Y-ohio* shows up-regulation of testis-specific gene expression in two wild-type backgrounds, a pattern that is abolished upon introduction of heterozygous loss-of-function (LoF) mutations for Su(*var*)205 and mod(*mdg4*). This highlights an attribute of YRV that is HP1- and Mod(*mdg4*)-dependent. Conversely, JIL-1 does not modify the pattern of YRV observed in the testis of wild-type genetic backgrounds. Taken together, the data suggest that the functions of HP1 and Mod(*mdg4*) contribute to Y-chromosome states that modify YRV in the *Drosophila* testis. The data also indicate that wild-type levels

of both Su(*var*)205 and mod(*mdg4*) products are required for the suppression of YRV in somatic tissues. Altogether, our observations suggest possibly adaptive and deleterious manifestations of cryptic polymorphic variation on highly heterochromatic *Drosophila* Y chromosomes.

Materials and Methods

Drosophila strains

Fly strains were obtained from the Bloomington Stock Center. The chromosomes containing the gene deletion of mod(*mdg4*)^{L3101} {y[1] w[1118]; P{w[+mC]=lacW}mod(*mdg4*) [L3101]/TM3, Ser[1]}, Su(*var*)205 {In(1)w[m4h]; Su(*var*)205[5]/In(2L)Cy, In(2R)Cy, Cy[1]}, mod(*mdg4*)^{G16853} {w[1118]; P{w[+mC]=EP}mod(*mdg4*) [G16853]/TM6C, Sb[1]}, and JIL-1^{Scim} {y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}JIL-1[Scim] ry[506]} were introgressed into the genetic background y[1]; bw[1]; e[4]; ci[1] ey[R] (Supporting Information, Figure S1, Figure S2, and Figure S3). All females used in the introgressions were collected within 7 hr after eclosion. For gene expression analyses, flies were grown in incubators at 25°, 65% of relative humidity, and constant light. Newly emerged adults harboring the mutations (Figure S2 and Figure S3) were collected and aged for 2 days under the same rearing condition before they were flash-frozen in liquid nitrogen. Four replicas of each sample were collected and stored at −80°. To assess PEV, male genotypes (*Y-ohio* or *Y-congo*) with loss-of-function mutations were crossed with virgin In(1)w[m4h]; Su(*var*)3-10[2]/TM3, Sb[1] Ser[1] or In(1)w[m4h]; Su(*var*)2-4[01] pie[54]/CyO. Flies showing variegated eyes were collected on the same day, aged for 24 hr, and flash-frozen in liquid nitrogen before pictures were taken.

Gene expression analysis

Microarrays were ~18,000-feature complementary DNA (cDNA) arrays spotted with *Drosophila melanogaster* cDNA PCR products. Total RNA was extracted from whole flies using TRIzol (Life Technologies). The synthesis of cDNA and its labeling with fluorescent dyes (Cy3 and Cy5) as well as hybridization conditions were carried out using 3DNA protocols and reagents (Genisphere). Four replicas of each sample were hybridized and scanned with Axon 400B scanner (Axon Instruments) and GenePix Pro 6.0 software. We used stringent quality control criteria to ensure reliability of foreground intensity reads for both Cy3 and Cy5 channels (Lemos *et al.* 2008). The procedure screens out poor quality arrays and ensures that the resulting data are minimally sensitive to the choice of normalization method. Foreground fluorescence of dye intensities was normalized by the Loess method in Bioconductor/Limma (Smyth and Speed 2003; Smyth 2005). The significance of variation in gene expression was assessed with the Bayesian analysis of gene expression levels (BAGEL) (Townsend and Hartl 2002). Results were checked for consistency using linear models in Limma.

False discovery rates (FDRs) were empirically estimated by permutation of the data set. The microarray gene expression data used here can be obtained at the Gene Expression Omnibus database under accession nos. GSE46313 and GSE9457. For quantitative PCR (qPCR) analyses, three biological replicates of 10 adult flies each were flash-frozen in liquid nitrogen. Total RNA was extracted with TRIzol (Life Technologies), and cDNA was synthesized with a QuantiTect Reverse-Transcription Kit (Qiagen). Real-time qPCR analyses were carried out with the Fast Sybr Green Master Mix (Applied Biosystems) using 7900HT Fast Real-Time PCR (Applied Biosystems). Expression level of the *Su(var)205* gene was analyzed with the software REST (Pfaffl *et al.* 2002).

Tissue expression

Tissue specificity of differentially expressed (DE) genes was assessed using the FlyAtlas (Chintapalli *et al.* 2007; downloaded on December 2011). We filtered the data to include only a nonredundant set of adult tissues: brain, eye, crop, midgut, hindgut, tubule, testis, accessory gland, adult fat body, heart, and trachea. For each Affymetrix probe in the FlyAtlas data set and for each tissue, we set expression level to 0 unless the probe was called as “present” in at least two of four arrays and then averaged over all probes and arrays for each FBgn to calculate the expression level for each gene in each tissue. Genes with expression counts below a threshold (20, 100, 250) were conservatively set to zero. We calculated a tissue prevalence index (TPi) for tissue *i* in a set of *n* tissues as:

$$TP(i) = \frac{\text{Number of DE genes with expression in tissue}(i)}{\text{Total number of DE genes}}$$

$TP(i)$ of 1 means that all genes in the set of differentially expressed genes show evidence of expression in the focal tissue.

Results

Su(var)205 and *mod(mdg4)* are dominant suppressors of PEV regardless of Y-chromosome background

To address the interaction between Y chromosomes and two key suppressors of variegation, we introgressed *Su(var)205* and *mod(mdg4)* LoF mutations into an isogenic genotype with variable Y chromosomes (Figure S1, Figure S2, and Figure S3). The two Y chromosomes assayed were chosen due to their contrasting contributions to *w(m4h)* variegation (Figure 1A; Lemos *et al.* 2010). We assayed for PEV in genotypes containing LoF mutations in *Su(var)205* or *mod(mdg4)* and differing only in the origin of the Y chromosome. As expected, we observed that the mutation in *Su(var)205* resulted in a wild-type eye phenotype in the background of both Y chromosomes (Figure 1B). Similarly, two LoF alleles of *mod(mdg4)* [alleles *mod(mdg4)^{L301}* and *mod(mdg4)^{G16853}*] resulted in a wild-type eye phenotype in the

Figure 1 Effects of chromatin modulators on white-mottled (*w(m4h)*) variegation in *Drosophila* genotypes with diverse Y chromosomes (A–E). *Su(var)205* (B) and two distinct alleles of *mod(mdg4)* (C, D) are strong suppressors of chromatin spreading and overcome the enhancer-of-variegation effect of the *Y-congo*. *JIL-1^{Scim}* (E) is a strong enhancer of variegation, which is effective even in the background of the *Y-ohio*.

background of both Y chromosomes (Figure 1, C and D). The *mod(mdg4)^{L301}* allele is a well-characterized allele with a P-element insertion in the third exon of the gene (Mongelard *et al.* 2002). Collectively, our results indicate that the enhancer-of-variegation strength of the *Y-congo* chromosome cannot counterbalance the suppression of variegation that is observed in genotypes that are heterozygous for a LoF mutation in *Su(var)205* or *mod(mdg4)*.

JIL-1 is a dominant enhancer of PEV regardless of Y-chromosome background

JIL-1 is a multidomain protein responsible for the phosphorylation of the histone H3, as well as maintenance of the structure of the polytene chromosomes in *Drosophila*. *JIL-1* LoF alleles have a strong effect on PEV, and *JIL-1; Su(var)205* and *JIL-1; Su(var)3-9* double mutants show enhanced variegation (Wang *et al.* 2011). This indicates that reduced dosage of *JIL-1* can counterbalance the typical suppressive effect of HP1 and *Su(var)3-9* on PEV. We tested whether *JIL-1* impacts PEV in the background of both *Y-congo* and *Y-ohio* genotypes. As in the experiment described above, the strains differed only in the origin of the Y chromosome and the presence of a chromosome containing a LoF *JIL-1* allele. We observed that both *Y-congo* and *Y-ohio* show a markedly white-eye phenotype in the heterozygous *JIL-1/+* genotype (Figure 1E). This indicates that loss of function of *JIL-1* has an enhancer-of-variegation effect that is independent of the Y-chromosome genetic background. This occurs in spite of

Figure 2 Y-linked regulatory variation in heterozygous genotypes with a loss-of-function *Su(var)205* mutation. (A) Similar number of genes differentially expressed (BPP > 0.99; FDR < 0.10) between *Y-congo* and *Y-ohio* when assayed in wild-type and *Su(var)205* loss-of-function genetic backgrounds. (B) qPCR indicates an ~50–60% reduction in *Su(var)205* mRNA abundance in *Su(var)205*/+ genotypes. (C) Volcano plot showing similarity between numbers and fold-changes of differentially expressed genes in the *Su(var)205*/+ background. Solid circles denote genes differentially expressed. Genes above and below zero are, respectively, up- and down-regulated in *Y-ohio*. (D) Agreement in fold-change estimates obtained with linear models in Limma and with Bayesian methods in BAGEL; data are for the contrast between *Su(var)205*/+; *Y-congo* and *Su(var)205*/+; *Y-ohio*.

the fact that the *JIL-1* allele tested is a weak hypomorphic allele (Zhang *et al.* 2003).

Su(var)205*-dependent up-regulation of testis-specific gene expression in *Y-ohio

HP1 is a major component of heterochromatin and one of the strongest known suppressors of variegation. Results presented thus far indicate that *Su(var)205* has an overwhelming suppressive contribution to PEV, which might completely mask the contribution of naturally occurring Y-chromosome variants. The possibility remained, however, that HP1 might modulate gene expression in a Y-chromosome-dependent manner. Using the FlyAtlas (Chintapalli *et al.* 2007), we observed that, in a wild-type genetic background, the *Y-ohio* leads to a substantial up-regulation of testis-specific gene expression relative to the *Y-congo* (% testis-specific across the genome, 14.6; % testis-specific up-regulated by *Y-ohio* in the wild-type background, 41.2; Fisher's exact test odds ratio, 2.82; $P = 1.56E-15$). Greater up-regulation of genes expressed in testis might be expected in *Y-ohio* because this Y-chromosome variant acts as a suppressor of variegation. However, when the whole genome is

considered, a similar number of genes appear up-regulated and down-regulated in *Y-ohio* (Figure 2A).

To address the hypothesis that *Y-ohio* up-regulation of testis-specific expression might be dependent on HP1 function, we profiled Y-linked regulatory variation in flies that were heterozygous for a LoF allele of the *Su(var)205* gene. The presence of one defective allele knocks down the expression of the *Su(var)205* gene significantly (Figure 2B). Thus, we contrasted *Su(var)205*/+; *Y-congo* and *Su(var)205*/+; *Y-ohio*, which are genetically identical except for Y-chromosome origin. First, we observed hundreds of genes significantly DE in this comparison, with the *Y-congo* and *Y-ohio* inducing similar numbers of genes in both the wild-type and *Su(var)205*/+ genotypes (Figure 2, A and C). We observed 473 DE genes at Bayesian posterior probability (BPP) > 0.99 (FDR < 0.10), with a comparable number of genes up- and down-regulated (Figure 2, A and C). We observed a high correlation between *P*-values obtained with linear models in Limma and BPP obtained in BAGEL ($\rho = 0.96$; $P < 10E-16$), with nearly perfect correspondence between the fold-change estimates by the two methods ($\rho = 0.99$; $P < E-16$; Figure 2D). Finally, we investigated the consequences of the *Su(var)205* LoF allele

Figure 3 Wild-type level of HP1 is required for Y chromosome-dependent up-regulation of testis-specific genes. Heat maps showing the genes (vertical columns) that are up-regulated in *Y-ohio* relative to *Y-congo*, with their expression values across 11 tissues (horizontal lines). Data are shown for genes that are up-regulated in *Y-ohio* relative to *Y-congo* in the *+/+* and *Su(var)205/+* genetic backgrounds. The scale color of the heat map was normalized for each gene with green and red denoting high and low transcript abundance, respectively. The first line in each of the heat maps depicts expression levels in the testis tissue. The evident green line, highlighted by the black bar on the top of the heat maps, indicates testis-specific genes that are up-regulated in *Y-ohio*.

on the patterns of testis-specific up-regulation that we observed in the *Y-ohio* background. Surprisingly, the Y-chromosome-dependent up-regulation of testis-specific genes in *Y-ohio* vanishes in the *Su(var)205/+* genotype (Figure 3). Indeed, the number of DE testis-specific genes in *Su(var)205/+* genotypes with variable Y chromosomes is actually significantly smaller than that expected from the genomic background (testis-specific genes genome-wide, 14.6%; testis-specific genes up-regulated by *Y-ohio* in the *Su(var)205/+* background, 6.2%; Fisher's exact test odds ratio, 0.41; $P = 0.0006$). Altogether, these data indicate that wild-type HP1 levels are required for the manifestation of testis-specific gene expression differences that are exerted by cryptic Y-chromosome polymorphisms.

YRV in genetic backgrounds with loss-of-function mutations in *mod(mdg4)* and *JIL-1*

Our analyses suggested a complex architecture of Y-chromosome-by-background interaction, with genomic background exerting a strong influence on YRV. To address the robustness in the pattern of testis-specific up-regulation in *Y-ohio*, we performed genome-wide expression analysis in additional backgrounds. As expected, in a second wild-type strain, *Y-ohio* showed the same pattern and a similar magnitude of testis-specific gene up-regulation as observed before (41.2% of the DE genes in background 1 are testis specific vs. 45.1% in background 2). To extrapolate the results obtained with *Su(var)205/+* to other chromatin regulators, we assessed the gene expression profile in *mod(mdg4)/+* and *JIL-1/+* heterozygous. Testis-specific up-regulation in *Y-ohio* is insensitive to the presence of a *JIL-1* LoF allele, with 49% of the genes up-regulated in *JIL-1/+*; *Y-ohio* showing testis specificity relative to 14.6% across the genome (Fisher's exact test odds ratio, 3.42; $P = 7.897E-10$;

Figure 4). Indeed, the proportion of genes up-regulated in *Y-ohio* that are testis specific is indistinguishable between the two wild-type backgrounds and the *JIL-1/+* genotype (Fisher's exact test, $P > 0.10$ in all comparisons between these three genotypes). Notably, this stability occurs in spite of the substantial effect of the *JIL-1* allele on PEV, which is indistinguishable between *Y-ohio* and *Y-congo* when these chromosomes are in the presence of a *JIL-1* LoF mutation (Figure 1E). On the other hand, results with *mod(mdg4)/+* recapitulate those observed with *Su(var)205/+*; namely, the pattern of *Y-ohio* testis-specific gene up-regulation vanishes (Figure 4).

Ubiquitously expressed genes are disrupted in *Su(var)205*-dependent and *mod(mdg4)*-dependent YRV

Genetic interactions between *Su(var)205* and *Y-ohio* and *mod(mdg4)* and *Y-ohio* are required to promote the *Y-ohio*-dependent up-regulation of gene expression in the testis (Figure 5). To further address the consequences of the breakdown of genetic interactions in *Su(var)205/+* genotypes, we investigated patterns of tissue up-regulation using the FlyAtlas data set (Chintapalli *et al.* 2007). FlyAtlas is a unique resource with tissue expression data collected across several tissues. To gauge the contribution of specific tissues to YRV, we devised a tissue prevalence index (TPI) to estimate the contribution of genes expressed in each tissue to the total number of DE genes. Figure 6A shows that the genes up-regulated in two wild-type backgrounds and the *JIL-1/+* genotype display a stable and coherent pattern: 44–58% of genes up-regulated in *Y-ohio* show evidence of testis expression above the detectable threshold. On the other hand, <18% of the genes up-regulated in *Y-ohio* show evidence of expression in any of the other tissues. Remarkably, the introduction of the *Su(var)205* or *mod(mdg4)* LoF allele

Figure 4 Genetic interaction between the *Y* chromosome and *mod(mdg4)* modulates testis-specific gene expression. Heat maps showing the genes (vertical columns) that are up-regulated in *Y-ohio* relative to *Y-congo* in the *+/+* (background 2), *mod(mdg4)/+*, and *JIL-1/+* genetic backgrounds with their expression values across 11 tissues (horizontal lines). The scale color of the heat map was normalized for each gene with green and red denoting high and low transcript abundance, respectively. The first line in each of the heat maps depicts expression levels in the testis tissue. The black bar on the top of the heat maps indicates testis-specific or testis-biased genes that are up-regulated in *Y-ohio*.

causes the differential expression of several genes that are expressed in somatic tissues while it masks the original differences detected in the testis (Figure 6B). Indeed, this is in agreement with our observations that genes differentially expressed in the *Su(var)205* background show a significant depletion in targets with testis expression (Figure 5). For the assessment of tissue prevalence, we used average expression counts >100 units across the four replicates in the FlyAtlas set to be an indication that the gene is expressed. Using more stringent (200 expression units) or less stringent (20 expression units) cutoff thresholds for ascertaining expression yielded nearly identical results. Hence, we conclude that the wild-type functions of *Su(var)205* and *mod(mdg4)* might be required to mitigate the impact of *Y*-chromosome polymorphisms on gene expression in somatic tissues.

Discussion

Chromatin modifications are central to genomic regulation, with euchromatin and heterochromatin as the most clearly distinguishable domains. Chromatin states are defined by post-transcriptional marks on histones that guide nonhistone proteins to bind specifically to the DNA in active or inactive segments of DNA. In *Drosophila*, heterochromatin represents 30–35% of the genome of most somatic cells (Girton and Johansen 2008), and the proteins *Su(var)3-9*, *Su(var)3-7*, and HP1 are abundant components of heterochromatin. The *Y* chromosome represents a major heterochromatin segment in fruit flies, and its content modulates PEV and gene expression (Dimitri and Pisano 1989; Ashburner *et al.* 2005; Lemos *et al.* 2008; Jiang *et al.* 2010; Lemos *et al.* 2010).

Interactions between *Su(var)205* and *YRV*

HP1 is a structural nonhistone chromosomal protein that is conserved from human to yeast (Saunders *et al.* 1993; Allshire *et al.* 1995; Pak *et al.* 1997). It functions in heterochromatin-mediated gene silencing, as well as in the regulation of active euchromatic genes (Eissenberg *et al.* 1990; de Wit *et al.* 2007; Kwon and Workman 2011; Yin *et al.* 2011). The epigenetic mark for HP1 recruitment and heterochromatin formation is H3K9 methylation, exerted by the enzyme *Su(var)3-9* (Rea *et al.* 2000; Peters *et al.* 2003). H3K9 methylation marks generate affinity sites for HP1, and interactions between HP1 and *Su(var)3-9* and between HP1 and *Su(var)3-7* are critical for the establishment of heterochromatin (Cleard *et al.* 1997; Bannister *et al.* 2001; Lachner *et al.* 2001; Delattre *et al.* 2004; Vermaak and Malik 2009). In *Drosophila*, the amount of HP1 correlates with suppression of the PEV phenotype (Eissenberg *et al.* 1990). As expected, heterozygous genotypes containing a LoF allele of *Su(var)205* show an ~50% decrease in mRNA abundance, leading to a strong suppression of heterochromatin spreading that shifts the PEV phenotype to wild type. We observed that the ability of HP1 to suppress PEV is independent of the presence of a strong naturally occurring enhancer-of-variegation *Y-congo* chromosome in the genetic background. On the other hand, gene expression analysis uncovered genetic interactions between HP1 dosage and polymorphic variation in *Y* chromosomes. Accordingly, the results indicate that polymorphic *Y*-chromosome-dependent modulation of testis-specific gene expression is modified by HP1 dosage. The affected pathways are unclear because the vast majority of testis-specific genes are yet to be

Figure 5 Proportion of testis-specific genes in the set of genes up-regulated by *Y-ohio* in the presence/absence of loss-of-function alleles. *Y-ohio* up-regulation of testis-specific expression is *Su(var)205*-dependent and *mod(mdg4)*-dependent. Two +/+ backgrounds were used (see Figure S1, Figure S2, and Figure S3 for details on backgrounds). *P*-values (based on Fisher's exact tests) relative to the proportion of testis-specific genes across the genome (14.6%) is <0.001 for all cases.

functionally characterized (McQuilton *et al.* 2012). Nevertheless, testis-specific genes are enriched in “black” chromatin (Filion *et al.* 2010), which is the prevalent type of repressive chromatin in the *Drosophila* embryonic cell line Kc167. Black chromatin is substantially marked by histone H1 and chromosomal proteins IAL, D1, and SUUR, whereas Su(Hw), LAM, and EFF are also frequently present (Filion *et al.* 2010). Finally, we emphasize that the effects described here are not attributable to the *Y* chromosome or to *Su(var)205* alone, but are instead dependent on the interaction between the *Su(var)205* locus and cryptic polymorphic factors on the *Y* chromosome.

Interactions between *mod(mdg4)* and YRV

Modifier of *mdg4* [Mod(*mdg4*)] is a multifunctional protein that encodes a BTB/POZ domain transcription factor (Read *et al.* 2000). Transcripts of this locus can be differentially processed and generate numerous splice variants, with a 2.2-kb transcript being the most abundant isoform (Gerasimova *et al.* 1995; Buchner *et al.* 2000; Labrador *et al.* 2001). The *mod(mdg4)* LoF allele used in this work [*mod(mdg4)*^{L3101}] is a recessive homozygous lethal in the larval stage. It is also a strong suppressor of *cycE* (Read *et al.* 2000), which may play an important role in the regulation and entry into the S phase of the cell cycle. Furthermore, mutations in *mod(mdg4)* disrupt X–Y segregation more severely than autosomal segregation (Soltani-Bejnood *et al.* 2007) and display a recessive-lethal interaction with the *Y* chromosome. Altogether, these results suggest that *mod(mdg4)* interacts with the *Y* chromosome. Here we observed that *mod(mdg4)* deficiency specifically disturbs the expression of testis-specific genes in *Y-ohio*. As noted, these genes

are abundant in black chromatin, which is frequently marked by the chromosomal proteins D1 and Su(Hw) (Filion *et al.* 2010). D1 and Su(Hw) proteins physically interact with Mod(*mdg4*) (Guruharsha *et al.* 2011), and Su(Hw) is essential for Mod(*mdg4*) function (Georgiev and Kozycina 1996; Gdula and Corces 1997). These observations raise the possibility that the pathways through which Mod(*mdg4*) interacts with the *Y* chromosome intersect those mediated by HP1, Su(Hw), and D1. Specifically, D1 binds to satellite DNA repeats (Blattes *et al.* 2006). Furthermore, the Su(Hw) protein is required for the gypsy transposon insulator to block enhancer–promoter interactions (Gause *et al.* 2001). Upon DNA binding, Su(Hw) recruits Mod(*mdg4*), and Mod(*mdg4*) determines how gypsy insertions alter gene expression with insulator and repressor functions (Gerasimova *et al.* 1995; Georgiev and Kozycina 1996; Cai and Levine 1997). Together, our results indicate that the interaction between Mod(*mdg4*) and the *Y* chromosome is required for testis-specific gene up-regulation in *Y-ohio*. The interactions might be mediated by cryptic factors that are polymorphic in the *Y* chromosome and affect spermatogenesis. The data also suggest that wild-type *mod(mdg4)* functions might be required to suppress the somatic effects of cryptic Y-linked polymorphisms.

Interactions between *JIL-1* and YRV

Many chromatin-remodeling proteins might be able to interact directly or indirectly with the *Y* chromosome and modulate PEV. Here we addressed whether reductions in *JIL-1* dosage due to heterozygous LoF mutations might be important modifiers of YRV. *JIL-1*, a kinase responsible for H3S10 phosphorylation, can reduce the suppression-of-PEV phenotype of *Su(var)3-9*, *Su(var)3-7*, and *Su(var)205* LoF mutations (Deng *et al.* 2010; Wang *et al.* 2011). We observed this same result when we introgressed *Y-congo* and *Y-ohio* into the *JIL-1*^{Scim} LoF allele. The variegated phenotype was abolished even for the *Y-ohio*, which shows a strong suppressor-of-variegation capacity. This occurs despite the fact that *JIL-1*^{Scim} express nearly 58% of native protein, while other alleles abolish a larger fraction of the protein pool (Zhang *et al.* 2003). We cannot exclude the possibility that stronger *JIL-1* LoF could disrupt testis-specific up-regulation in *Y-ohio*. It is also possible that over evolutionary timescales, subtle interactions between *JIL-1* alleles and variable *Y* chromosomes play important roles in the dynamics of chromatin states in natural populations.

Evolution of *Y*-chromosome-by-autosome interactions

A key puzzle is the interplay between dosage variation in chromatin components and variable *Y*-chromosome chromatin. Polymorphic *Y* chromosomes might interact with naturally occurring variation in chromatin dosage across individual genotypes and tissue types. Our findings point to interactions between the sources of YRV in *Y* chromosomes and the dosage of HP1 and Mod(*mdg4*). Disruption in backgrounds with mutations in *Su(var)205* or *mod(mdg4)*

Figure 6 Tissue prevalence of genes that are modulated by genetic interactions between Y chromosomes and *Su(var)205*, *mod(mdg4)*, and *Jil-1*. (A) The contrasts between *Y-congo* and *Y-ohio* in two control wild-type *+/+* genotypes and in the *Jil-1/+* genotype indicate that the majority of the genes up-regulated by *Y-ohio* show evidence for expression in the testis. (B) The contrasts between *Y-congo* and *Y-ohio* in the *Su(var)205/+* and *mod(mdg4)/+* genotypes indicate that the genes up-regulated in *Y-ohio* show a prevalence for expression in somatic tissues. For reference, a wild type *+/+* is also displayed in B.

leads to the tissue-wide consequences of YRV. One hypothesis is that YRV in somatic tissues might be deleterious, with limited fitness benefits accrued from the interaction between Y chromosomes and variable chromatin dosage. Indeed, we expect that tissue-wide disruption of gene expression due to the interaction between *Su(var)205* and *mod(mdg4)* with polymorphic Y chromosomes might be deleterious over evolutionary timescales. On the other hand, testis-specific YRV might be conditionally beneficial and driven by adaptive Y-chromosome evolution and positive natural selection of cryptic factors responding to HP1 levels. In this notion, the higher expression of testis-specific genes that is driven by the Y chromosome in specific backgrounds might result in adaptive variation in reproductive fitness. Accordingly, evidence that polymorphic Y chromosomes interact with the autosomal background and impact reproductive phenotypes and fitness has been documented (Chippindale and Rice 2001). Interestingly, the higher rDNA copy number in *Y-congo* relative to *Y-ohio* (Paredes *et al.* 2011) might suggest a role for rDNA–HP1 and rDNA–Mod(mdg4) interactions in fitness. We predict that the balance between somatic and testis-specific consequences of YRV in variable genomic backgrounds encountered in natural populations will prove to be a major determinant of how natural selection shapes the evolution of the Y chromosome and its repeats.

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Supporting Information

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Chromatin-Associated Proteins HP1 and Mod(mdg4) Modify Y-Linked Regulatory Variation in the *Drosophila* Testis

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Figure S1 Crosses to obtain Y chromosome substitutions lines. Chromosomes in the recipient marked strain are shown in black. Chromosomes in the donor strain are shown in gray.

Figure S2 Chromosome introgressions and genotypes contrasted by microarray. The *Y-ohio* is shown in red, the *Y-congo* is shown in green, and the chromosome harboring the *Su(var)205* loss-of-function allele is shown in yellow. Note that all genotypes contrasted differ only in the origin of the *Y* chromosomes (*Y-ohio* vs *Y-congo*). The genetic background is denoted “background 1”.

Figure S3 Chromosome introgressions and genotypes contrasted by microarray. The *Y-ohio* is shown in red, the *Y-congo* is shown in green, and the chromosomes harboring the *mod(mdg4)* or *JIL-1* loss-of-function alleles are shown in yellow. Note that all genotypes contrasted differ only in the origin of the Y chromosomes (*Y-ohio* vs *Y-congo*). The genetic background is denoted “background 2”.