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Role of Rapgap1 in \textit{Sxl} Activation in \textit{Drosophila melanogaster}

Katie Barnes

Abstract

The master switch of the sexual differentiation and dosage compensation pathway in \textit{Drosophila} is the sex lethal gene, \textit{Sxl}. The early promoter, \textit{Sxl}_{Pe}, is activated in females, resulting in female-specific splicing of later transcripts (notably the late \textit{Sxl} transcript \textit{Sxl}_{Pm}), while inactive in males. Chromatin immunoprecipitation (ChIP) assays have previously shown association of two conventionally heterochromatin-localized proteins, HOAP and HP1, at \textit{Sxl}_{Pe}, and \textit{in situ} hybridization as well as RT-PCR assays have confirmed a repressive role for HOAP and both repressive and activating roles for HP1. The mechanism for the activity shift of HP1 is currently unknown. Deletions in the region of \textit{Rapgap1} yielded a similar phenotype to that of HP1 mutants, suggesting a potential role in the regulation of its activity. Attempted PCR characterization of the deletion spans in the studied mutant strains, \textit{Rapgap1}^{22} and \textit{Rapgap1}^{47}, was unclear, leading to the redesign of chosen oligonucleotide primer pairs and protocol. Future analyses will include genetic crosses between Rapgap1 and HP1 mutants and RT-PCR assays to observe Rapgap1 interactions with HP1 and \textit{Sxl}.

Introduction

In order to understand the study and potential role of Rapgap1 in the sexual differentiation of \textit{Drosophila melanogaster}, much background is needed. In the \textit{Drosophila}, the principal regulatory gene that determines the pathway engaged in sexual differentiation is the sex lethal gene (\textit{Sxl}), and its regulation occurs at the early promoter (\textit{Sxl}_{Pe}). The activation of the early promoter results in the production of early \textit{Sxl} protein (Keyes, Cline,
This protein regulates splicing of later products of the gene as well as other genes upstream in the sexual differentiation pathway, which are necessary for functions such as proper dosage compensation and sexual development. Specifically, the early Sxl protein regulates the splicing of the Sxl RNA produced later in embryogenesis to exclude exon 3, which contains an in-frame stop codon, to yield a functional Sxl protein (Sxlpf; female splicing mode). This late Sxl protein is then able to direct further sex-specific splicing interactions. In males, the early promoter remains inactive. Therefore, exon 3 is included in the Sxl RNA, and translation produces a nonfunctional protein (male splicing mode).

Whether Sxlpf is activated is based upon the dosage of the X chromosome (King et al., 1995). As in humans, females have two X chromosomes (XX), while males have one X and one Y (XY). However, the Y chromosome is only necessary for male fertility and does not influence sexual determination, as it does in humans. The X chromosome encodes Sisterless (Sis) proteins, which form heterodimers with the maternal product Daughterless (Da) and act as activating factors. On the other hand, the autosomal-encoded protein Deadpan (Dpn) and maternal product Extramacrochaete (Emc) form heterodimers with Sis, preventing it from forming Da-Sis heterodimers and acting as repressors. Whether the Dpn or Sis proteins are able to bind to the early promoter is determined by the ratio of sex chromosomes (X) to autosomal chromosomes (A) in the embryo (X:A). Males, with only one X chromosome, have a ratio of 0.5, while females have a ratio of 1.0. Therefore, only females have a high enough relative amount of Sis to bind all of the Dpn and Emc factors and form enough Da-Sis heterodimers to facilitate the binding of these positive regulatory proteins over the negative ones (Dpn) and activate Sxlpf. While these factors influence regulation of the sex lethal early promoter, the entire mechanism of activation is not known. But, Dr. Kellum and associates
at the University of Kentucky have suggested a more complex mechanism involving two more proteins: Heterochromatin protein 1 (HP1) and HP1/origin recognition complex-associated protein (HOAP) (Li et al., 2011).

The genome of eukaryotes is comprised of two types of chromatin—euchromatin and heterochromatin. Euchromatin is gene rich and transcriptionally competent, while heterochromatin remains condensed, is relatively gene poor, and transcriptionally inert (Ris & Korenberg, 1979). HP1 was first discovered as a non-histone chromosomal protein on immunostained polytene chromosomes in heterochromatin, concentrated in centromeres (James & Elgin, 1986). The HP1 protein is encoded by the gene *Su(var)205*, and a mutation to the gene, and thus HP1, resulted in dominant and dosage-dependent suppression of position-effect variegation (PEV), demonstrating a strong role for HP1 and potentially other heterochromatin proteins in the heterochromatin-induced silencing of euchromatic genes (Eissenberg et al. 1990; Eissenberg, Hartnett, Reuter, & Morris, 1992). This and future research has shown HP1 to be involved in the formation of heterochromatin and telomere capping, acting to silence genes (Kellum, 2003; Li et al., 2011 and references therein). However, it has also been found in euchromatin, though its corresponding roles are not as well understood.

HP1 has at least three different isoforms in *Drosophila*: HP1a, b, and c (Kwon and Workman, 2011 and references therein). Two domains are highly conserved. These include an N-terminal chromo (chromatin organization modifier) domain (CD) for chromatin binding, specifically an affinity for methylated lysine 9 on histone H3 (MeH3K9; a modification catalyzed by the histone methyltransferase protein Su(var)3-9), and a C-terminal chromo shadow domain (CSD) for protein-protein interaction, including self-
association. These are separated by a variable hinge domain, rich in serine and threonine residues (Kellum 2003 and references therein). The functional equivalent of HP1 in budding yeast, Sir proteins are also capable of interacting with other proteins as well as each other and form a complex, which allows the formation of a domain of silenced chromatin (heterochromatin). As these domains provide HP1 with similar activity, it could function by a similar mechanism to repress gene expression and form heterochromatin in other eukaryotes. Its gene silencing activity is demonstrated by the complete lack of heterochromatic silencing in Su(var)205 mutants (Eissenberg & Hilliker, 2000).

While the key domains of the HP1 isoforms are conserved, they exhibit different functions and/or localization. Polytene chromosome preparations revealed that HP1a and HP1b are found primarily in heterochromatin, while HP1c is found in euchromatin (Kwon & Workman, 2011 and references therein). The differential localization of HP1 suggests that HP1 is also involved in gene regulation in euchromatin, though these roles have not been clearly studied and defined. Furthermore, while it is known that HP1 plays a role in repression and silencing genes, more recent studies have indicated activation roles for it as well. One Su(var)205 mutant produces variegated expression of light, and combinations downregulate the expression of light and rolled (Eissenberg & Hilliker, 2000 and references therein). Further evidence of HP1 involvement in the positive regulation of gene expression includes the discovered association of HP1c with RNA Pol II during transcriptional elongation (Kwon & Workman, 2011). Cryderman et al. compared mRNAs from wildtype and Su(var)205 mutant Drosophila, discovering that HP1 is specifically necessary for the expression of genes Pros35, CG5676, and cdc2 (2005). The differential localization of the HP1 isoforms further suggest isoform-specific function of the protein, which could provide
clues to characteristics or regulation factors of HP1 that produce either a repressive or activating function.

Regulation of HP1 function may be due to post-translational modifications (PTMs), such as methylation, acetylation, phosphorylation, etc. Some of the serine and threonine residues within the variable hinge domain of HP1 isoforms act as phosphorylation sites (Kellum, 2003). Evidence of regulation via phosphorylation includes the action of a hyperphosphorylated form of HP1a in the formation of heterochromatin, yet, when hypophosphorylated, HP1 associates with the origin recognition complex and interphase chromosomes (Kwon & Workman, 2011). Silencing activity, heterochromatin assembly, and protein interactions have been correlated with phosphorylation of HP1 (Kellum, 2003 and references therein). The altered interactions of HP1 in differing phosphorylated states suggest that its function is at least in part determined by phosphorylation, which may be notable later in the study of Rapgap1 in relation to HP1 and activation of SxlRNA.

HP1 targeting to heterochromatic sites appears to occur through association with origin recognition complex (ORC) proteins as well as an HP1/ORC-associated protein (HOAP), interacting with its C-terminus CSD (Kellum, 2003; Kwon & Workman, 2011). HP1a in Drosophila binds to ORC1-6, which may recruit silencing proteins to induce heterochromatic silencing. HOAP also appears at these sites, and mutations to ORC and HOAP suppress PEV, supporting roles in heterochromatin-induced gene repression (Kwon & Workman, 2011). HOAP is primarily found in conjunction with HP1 at telomeres, involved in telomere capping. However, it is also found in multiple other heterochromatic and euchromatic sites, as seen in immunostained larval salivary gland polytene chromosomes (Li et al., 2011 and references therein).
Microarray expression profiling was employed to study non-telomeric functions of HOAP, profiling a mutant to distinguish potential HOAP-regulated genes. First of all, the affected genes exhibited lowered expression, rather than the expected elevation due to HOAP’s identification as a repressor protein. Of the genes displaying reduced expression, most are only expressed in the testis. Therefore, consequences on sex ratios were observed. The resulting female-to-male sex ratio of the offspring having a null allele of HOAP (cav1) RNAi knockdown of HOAP (as well as HP1), and a newly recovered dominant negative allele (cav2248) was approximately 2:1. This reduction in male viability suggests a role for HOAP and HP1 at the promoter of the master sex determination gene, Sxl (Li et al., 2011).

Figure 1A displays the results of a subsequent cross of cav2248 mutants, which yielded a significantly increased female-to-male sex ratio due to reduced male viability, supporting the role of HOAP as a repressor. When combined with a Sxl deficiency (Sxl-), SxlP1, a rescuing effect occurred, restoring a proper ratio of approximately 1:1. This was expected, as inappropriate activation of Sxl should be countered by a deleterious mutation in the gene. To further explore the effects of HP1, crosses were performed combining mutations in Su(var)205—Su(var)2055, Su(var)2054, and Su(var)2052—with Sxl (Figure 1B). Su(var)2055 is a null allele, Su(var)2054 is a carboxyl-terminally deleted allele, and the Su(var)2052 allele contains a point mutation in the MeH3K9-binding CD. The combined maternal Su(var)2055 and paternal Sxl- mutations yielded the most sizeable effect, significantly reducing female viability. Su(var)2054 also produced a significantly lowered female-to-male ratio, and Su(var)2052 produced a moderate similar effect. Reduced female viability suggests a strong role for HP1 in the activation of Sxl. A reciprocal cross was performed, yielding no significant effect. Therefore, the mutations in HOAP and HP1 exhibit
Figure 1. Effects of mutations in HOAP, HP1, and Sxl on the sex ratios of *Drosophila* progeny.

A) The heterozygous *cav<sup>2248</sup>* progeny exhibit a significantly altered female to male ratio, indicating male viability (p<0.05). The addition of a mutant *Sxl* allele, however, had a rescuing effect, returning an expected ratio.

B) Maternal *Su(var)205* mutations were added to paternal *Sxl* mutations, resulting in reduced female viability. The balancer mutation Curly-O (CyO) was utilized to differentiate between the offspring with the HP1 mutation and those without. *Su(var)205<sup>5</sup>* yielded the most significantly altered sex ratio, with *Su(var)205<sup>4</sup>* producing an intermediate one and *Su(var)205<sup>2</sup>* producing a small, statistically insignificant shift, and was only combined with the *Sxl<sup>FP780</sup>* allele. The third cross tested the effect of a paternal mutation of *Su(var)205*, which produced no significant effect. Thus, there is strictly a maternal effect.

C) These crosses tested the effect of combining the *Su(var)205<sup>5</sup>* mutation with HOAP mutants *ca<sup>v</sup>2248* and *ca<sup>v</sup>1*, from both the mother and father. The maternal *ca<sup>v</sup>* mutations rescued the skewed ratio due to female lethality from the *Su(var)* mutation, while the paternal mutation failed to. (p<0.05**, p<0.10*; From Li et al., 2011)
a maternal effect. Finally, neither maternal mutation $cav^4$ nor $cav^{2248}$, when crossed with $Sxl^-$ fathers produced a significant effect on the sex ratio or, therefore, either sex’s viability (Figure 1C). However, when added to maternal $Su(var)205^5$, HOAP mutants rescued the reduced female viability effect, restoring an insignificantly altered ratio. This effect only occurred when $Su(var)205^5$ came from the mother, also exhibiting a maternal effect.

In order to observe direct effects on expression of the sex lethal gene, sequences of the late transcript ($Sxl_{pm}$) were tagged to indicate male-specific and female-specific splicing patterns using reverse transcriptase polymerase chain reaction (RT-PCR) assays (Figure 2). HOAP mutant males ($cav^4$ or $cav^{2248}$) exhibited evidence of female-specific bands (short P1/P3 transcripts), indicating the inappropriate activation and firing of $Sxl_{pe}$ in males and supporting a repressive role for HOAP. In HP1 mutants, with the $Su(var)205^4$ or $Su(var)205^5$ allele, males also exhibited the female-specific band, advocating repressor activity for HP1. However, male-specific bands (P2/P3 transcripts) also appeared in females, revealing that the early promoter most likely was not activated, indicating another role for HP1 in activation.

To pinpoint influenced activity of reduced HOAP and HP1 to the early promoter of the sex lethal gene, in situ hybridizations were performed, allowing the visualization of $Sxl_{pe}$ transcripts in 0-4 hr embryos (Figure 3). Reduced HOAP resulted in earlier expression and increased expression of transcripts, indicating inappropriate expression in males. This increased activation confirms a repressive role for HOAP at the early promoter. On the other hand, reduced HP1 produces greatly reduced expression, revealing its activation role at the early promoter.
Figure 2. Effects on expressed sex-specific $SxLPm$ transcripts in HOAP and HP1 mutants from RT-PCR assays. A) Primers were used to identify male-specific and female specific transcripts in each sex. P1 identifies the transcribed 5th exon of the sex lethal gene; P2 signals expression of the 3rd, and P3 signals the 2nd. As mentioned earlier, in developing females, $SxLPe$ is activated and the 3rd exon is excluded, contrary to the male-specific pathway and transcript. B) Therefore, wildtype males have longer P1/P3 PCR fragments, as their transcripts include P2, and wildtype females display shorter PCR fragments and no evidence of a P2/P3 segment. However, $cav^1$ and $cav^{2248}$ mutant males show evidence of shorter, female-specific P1/P3 bands, showing that transcripts were inappropriately spliced ($SxLPe$ was inappropriately fired) in males. This finding suggests that HOAP acts as a repressor of expression of the sex lethal gene. C) The $Su(var)205$ mutant males show evidence of this shorter P1/P3 band as well, also indicating repressor activity for HP1. However, the RT-PCR results for the P2/P3 PCR product shows the presence of the male-specific band in females, indicating an activation role for HP1 also. This suggests that, during activation and transcription of sex lethal, HP1 may first act as a repressor with HOAP and then shift to an activating role. (From Li et al., 2011)
Figure 3. SxlPe in situ hybridizations of wildtype, cav2248, and Su(var)2055 embryos. In situ hybridizations were performed to directly visualize the transcription of the sex lethal early promoter in HOAP and HP1 mutants, compared to wildtype. Two transcripts, observed as dots, appear in females, and one in males (when inappropriately expressed). A) The first column shows the typical expression in wild type embryos, with transcripts appearing in females during cycle 12. The loss of function of HOAP resulted in earlier-appearing and an increased intensity of transcripts in females, verifying a repressive role for it at the early promoter. B) In contrast, loss of HP1 caused delayed and greatly reduced expression of early promoter transcripts, and as seen previously, increased female lethality. This finding confirms that HP1 acts as an activator at SxlPe (From Li et al., 2011)

The next step involved performing chromatin immunoprecipitation (ChIP) assays to determine whether the influences of HP1 and HOAP on regulation of SxlPe are due to physical associations with the locus (Figure 4). Both HP1 and HOAP are present at the sex lethal early promoter in early embryos, indicating that their regulation functions are most likely due to physical association. HOAP also appears at other loci within the gene, corresponding with positive-regulation binding sites and opposite the negative-regulation binding sites. This pattern suggests that the repressive role of HOAP could involve competing for binding sites for activating proteins, while allowing other repressive regulators to bind.
Figure 4. Chromatin immunoprecipitation (ChIP) assays of HOAP and HP1 at the \( Sx\ell \) locus. Enrichment of HOAP (red) and HP1 (blue) is plotted on a molecular map of the \( Sx\ell \) locus using 1-3 hour embryos. Indicated below the ChIP map is a diagram of E-box binding sites for positive regulatory factors (blue diamonds) and negative regulatory factors (red squares). Below that, another map displays the corresponding locations of the \( Sx\ell \) transcripts produced. Both HOAP and HP1 appear at \( Sx\ell_{Pe} \), HOAP also appearing in other locations on the gene. HOAP appears to bind in between negative E-box binding sites and correlate with the positive ones. (From Li et al., 2011)

Figure 5 portrays a proposed model for the mechanism of HOAP and HP1 action at the early promoter. Initially, both proteins are bound to the promoter, forming a repressive complex. This arrangement could prevent the random activation of the promoter and require a verified one-to-one X:A for normal activation in females. In males, HOAP remains, and Dpn is allowed to bind to the surrounding negative regulatory binding sites. The higher amount of the positive-regulatory Sis proteins in females allows them and Sis-Da heterodimers to displace HOAP and bind while HP1 remains, now contributing to the activation of \( Sx\ell_{Pe} \). How this shift in HP1 activity from repression to activation occurs is not currently known.
Figure 5. Prospective model of the interactions between HOAP and HP1 at Sxl. Before activation, HOAP is bound to HP1, and the repressor complex is bound to the promoter, inhibiting indiscriminate activation. In males, Dpn binds to the promoter, further repressing expression. In females, however, Sis proteins and Sis-Da heterodimers bind, kicking HOAP off. HP1 remains, now acting as an activator of expression. (From Li et al., 2011)

Experimental Methods

Experimental crosses were set up according to the same genetic assay that was used to identify the roles of HP1 and HOAP at the Sxl locus: CyO/Del ♂ x Sxl/Y. Various deletions along the second chromosome were used to investigate the presence of other proteins potentially influencing Sxl and/or the function of HP1. Once a similar sex ratio effect to that of HP1 mutants was observed, multiple smaller deletion variations were tested to narrow the region maintaining the effect toward a potential gene of interest. Specific mutants for that gene were then obtained and the sex ratio effects confirmed (the creation of the mutations is described in Chen, Barkett, Ram, Quintanilla, & Hariharan, 1997).
To characterize the deletions in the mutants, polymerase chain reaction (PCR) assays were used. A small number of adult flies were ground up, and genomic DNA was separated out from the wild type and both mutant strains. Oligonucleotide primers were designed to indicate the presence of 5′, middle, and 3′ segments of the gene of interest as well as sequences from a few flanking genes by producing products of approximately 500 base pairs (bp). After PCR amplification, a sample of each DNA-primer mix was transferred into a well of a 1.5% agarose gel in 1x TAE buffer, and gel electrophoresis was run to separate potential product bands.

Results

In an effort to identify other potential factors involved in the regulation of \( Sxl \) and, therefore, possibly HP1 activity, crosses were set up with varying deletions along the 2\(^{nd} \) chromosome, and the sex ratios of the offspring were observed to identify those with effects similar to that of HP1 mutations, which could suggest an interaction. Figure 6 displays a region that, when deleted, produced reduced female viability. From the tested deletions, the region yielding the effect has been narrowed down essentially to the \( Rapgap1 \) gene. Thus, two \( Rapgap1 \)-specific loss of function mutant strains were obtained, \( Rapgap1^{22} \) and \( Rapgap1^{47} \), and confirmation crosses were performed. Both mutations still resulted in reduced female viability (Table 1). However, these mutations were created using P-element-mediated mutagenesis, resulting in deletions in the \( Rapgap1 \) region (Chen et al., 1997). While the deletions were focused around \( Rapgap1 \), the exact span in these mutants was never characterized.
Figure 6. Genetic Interactor Map of 28A3-28B1 Region. This genetic map illustrates a deleted region that yielded a similar effect to HP1 mutants of reduced female viability. The tested deletions, shown in the lower portion in red, either exhibited an altered sex ratio (designated with a plus) or did not (designated with an X), and the resulting ratios are shown in Table 1. The deletion maintaining the effect has been narrowed down to the region of the Rapgap1 gene, between deletions 7804 and 108.

Table 1. Regional deletions and resulting sex ratios of progeny containing them.

<table>
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<tr>
<th>Region</th>
<th>Del/+ Female</th>
<th>Del/+ Male</th>
<th>CyO/+ Female</th>
<th>CyO/+ Male</th>
<th>Male:Female</th>
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<td>Del/+ Male</td>
<td>CyO/+ Female</td>
<td>CyO/+ Male</td>
<td>Male:Female</td>
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<tr>
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<td>24</td>
<td>20</td>
<td>19</td>
<td>3.4:1</td>
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<td>24</td>
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<td>22</td>
<td>49</td>
<td>1.9:1</td>
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<td>34</td>
<td>116</td>
<td>57</td>
<td>106</td>
<td>3.4:1</td>
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<tr>
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<td>52</td>
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<td>41</td>
<td>1.7:1</td>
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<tr>
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<td>25</td>
<td>43</td>
<td>34</td>
<td>37</td>
<td>1.7:1</td>
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<td>140</td>
<td></td>
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</table>

The resulting offspring containing deletions from Figure 6 and calculated male-to-female sex ratios. The deletion maintaining the effect was narrowed down to the region of Rapgap1. Two Rapgap1-specific mutant strains still exhibited reduced female viability.
Figure 7. Genetic map of the *Rapgap1* region and relative oligonucleotide primer locations. Oligonucleotide primer pairs were designed for the 5’, middle, and 3’ regions of *Rapgap1* as well as a few flanking genes: *Ziz*, *Obp28a*, and *CG6739*. A HOAP primer pair was also used as a control. Primer pairs were designed to yield products of approximately 500 base pairs in length for ease of distinction with gel electrophoresis.

Therefore, PCR assays were performed, using oligonucleotide primer pairs to signal the presence of sequences along the *Rapgap1* gene as well as in a few surrounding genes (Figure 7). Gel electrophoresis was utilized to separate and view the products (Figure 8). HOAP oligonucleotides were used as a control, forming a near 500-bp product band when paired with wild type DNA. The PCR products from each of the other primer pairs are expected to be of similar length, but similar sized products only appeared in the wild type DNA gel in the Obp28a, Rapgap-3’, and CG6739 lanes, and other smaller sized products were observed, calling into question the purity of the samples or protocol followed. The expected product bands also did not appear in the wild type samples with the *Ziz*, Rapgap-m, or Rapgap-5’ primer pairs, indicating that they may not be reliable sequences, so those will be redesigned. While there are still noticeable differences between the wild type and mutant strains, conclusions are being withheld until we the reaction conditions have been perfected.
Figure 8. PCR characterization of Rapgap1 mutants. Gel electrophoresis was used to separate and identify product bands from each PCR reaction. A gel was run for each mutant genome as well as a control using wild type DNA, with each included oligonucleotide pair labelled above its reaction lane. The HOAP + wild type DNA control reaction displays the expected, approximately 500-bp product band. This band also appears in the Obp28a, Rapgap-3', and CG6739 lanes, indicating functional primer pairs. However, they do not appear in the Ziz, Rapgap-m, or Rapgap-5' oligo pair lanes, and many extraneous bands are present. The Rapgap1^{22} and Rapgap1^{47} gels appear noticeably different, but we are refraining from drawing conclusions until the reaction conditions are improved.

Discussion

HP1 and HOAP are primarily associated with heterochromatin and involved in repressing roles. However, they have been tagged at various euchromatic sites, one of which has been discovered at the Sxl/early promoter. While HOAP maintains a repressive role, HP1 is also required for activation in females. However, this requires a shift in its activity, the mechanism of which is not known. A deletion on the second chromosome maintaining a similar effect to HP1 mutants alluded to a potential role for Rapgap1 in regulating this shift or, at least, another activation role at SxlPe. Unlike HP1 and HOAP mutants, the Drosophila carrying the Rapgap1^{22} and Rapgap1^{47} mutations survived to adulthood as homozygous mutants and altered sex ratios to a smaller degree, suggesting a more minor role. The Rapgap1-specific mutant strains obtained to study the direct effect of Rapgap1, however, are uncharacterized deletions. PCR characterization of the Rapgap1^{22} and Rapgap1^{47} mutants lacked product bands from some of the designed oligonucleotide primer pairs with the wild
type DNA, and many extraneous bands were present. Therefore, the Ziz, middle Rapgap1, and 5' Rapgap1 sequences will be redesigned, and alterations to protocols will be tested. Once the technique has been perfected, conclusions will be drawn.

Rapgap1 has already been discovered to exhibit GTPase activator activity in vitro, an activity that is needed for the inactivation of monomeric G proteins (Chen et al., 1997). Its transcripts are also specifically localized in the germ plasm of the developing embryo and found in mesenchyme cells during gastrulation, suggesting a role in differentiation (Wang, Khan, & Wieschaus, 2013). It could potentially play a role in switching HP1 into an activation state through its GTPase activator activity, which could aid in the phosphorylation or dephosphorylation of HP1, previously shown to alter its activity.

For future study of the potential interactions between the Rapgap1 and HP1 proteins, genetic crosses have been set up between the Rapgap1 and the weaker Su(var)2054 mutants. Resulting sex ratios will be recorded and analyzed for intensifying or rescuing effects to provide evidence of and insight into the nature of an interaction. The Rapgap1 mutations may also be added to ca\nu^{2248} to observe any interaction effects with HOAP. Finally, to determine whether the reduced female viability seen in Rapgap1 mutants is related to Sxl activity, RT-PCR assays will be performed to observe the direct effects of Rapgap1 on SxlPm transcripts. If further analysis is needed, in situ hybridizations showing direct SxlPe expression will also be performed.
References


