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Examining a Functional Interaction Between Chromatin Remodeler CHD1 and Histone H1 in D. melanogaster

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Examining a Functional Interaction Between Chromatin Remodeler CHD1 and Histone H1 in *D. melanogaster*

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By

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Abstract

Chromodomain-helicase-DNA-binding-protein-1 (CHD1) is a highly conserved ATP-dependent remodeling protein. It is localized to active genes and directs nucleosome spacing, while its loss has been linked to various human diseases, such as human prostate cancer. In *Drosophila*, CHD1 is important for fertility and wing development, and overexpression of CHD1 leads to severe wing vein defect phenotypes. The Linker Histone H1, which is known for maintaining heterochromatin and is associated with inactive genes, had been previously identified as a possible functional partner of CHD1, though the exact nature of their interaction is unclear. I undertook a genetic approach to examining the interaction between CHD1 and H1, making use of a novel genetic assay that had been previously developed in the Armstrong lab. This genetic assay uses the wing vein defects caused by CHD1 overexpression to identify factors that influence CHD1 function. I observed that CHD1 overexpression with the simultaneous knockdown of H1 resulted in an increase in the severity of wing vein defects, leading me to refine our working model for CHD1 and H1 interactions. Our working model suggests that CHD1 and H1 work competitively towards each other, with the absence of H1 allowing for increased CHD1 binding.

Introduction

Chromatin structure and remodeling

Eukaryotic genomes are condensed into chromatin, which is important for gene expression and regulation. Chromatin is made up of basic structural units called nucleosomes, which consist of 145-147 base pairs of DNA wrapped around an octamer of two copies of each of the four core histones (H3, H4, H2A, and H2B) (Zhou et al., 2018). Histones have short N-terminal tails (15-40 base pairs) that extend from the octamer and have been found to be the target of a number of post-translational modifications (Smith and Petersen, 2005). Histone H1, previously known as Linker Histone H1, has long been understood to be a structural component of chromatin, interacting with linker DNA entrance and exit sites between the nucleosomes (Widom, 1998).

Broadly, chromatin either exists as euchromatin—an "open" conformation associated with transcriptional activity—or heterochromatin—a "closed" form, associated with gene silencing. However, chromatin structure is also highly dynamic, having crucial impacts on gene expression and regulation by regulating gene accessibility. The main factors that determine whether chromatin is in its open or closed state are chromatin regulators. These chromatin regulators can be divided into two categories: (1) histone-modifying enzymes and (2) chromatin remodeling factors. Histone-modifying enzymes act through methylation, acetylation, phosphorylation, adenosine diphosphate–ribosylation, glycosylation, sumoylation, or ubiquitylation of histones. Chromatin remodeling factors alter the structure of position of the nucleosome using energy from ATP hydrolysis (Zhang et al., 2016).

CHD1 structure and function

CHD1 (Chromodomain-helicase-DNA-binding-protein-1) is an ATP dependent chromatin remodeler that is highly conserved from *Drosophila melanogaster* to humans (Smolle et al., 2012). CHD proteins are members of the SNF2 superfamily of chromatin remodelers. The CHD family is specifically characterized by the presence of an SNF2-related ATPase domain located in the central region of the protein, and two tandem chromodomains (Marfella and Imbalzano, 2007). The SNF2-like ATPase domain contains a conserved sequence of amino acids that has been found in proteins that are involved in cellular processes such as chromatin assembly, transcription regulation, and DNA replication (Smith and Petersen, 2005). The chromodomain (chromatin organization modifier domain) can mediate binding to histone residues, DNA, or RNA (Micuicci et al., 2015). CHD1 specifically contains the SANT and SLIDE DNA-binding domains, a bilobal motor domain that hydrolyses adenosine triphosphate ATP, and a regulatory double chromodomain (Fargung et al., 2017). CHD1 contacts the nucleosome at linker DNA via the DNA binding domains, and ATPase lobes (Sundaramoorthy et al., 2018).

Mutations in genes encoding various CHD proteins have been associated with various diseases and conditions in humans (Marfella and Imbalazo, 2007). Notably, CHD5 has been associated with neuroblastoma, a malignant neoplasm of the nervous system (White et al., 2005). Meanwhile, a sequence analysis of samples extracted from affected patients associated CHARGE syndrome with a loss of CHD7 (Vissers et al., 2004). The observation of an interaction between CHD3 and Ki-1/57, an intracellular phospo-protein involved in detecting malignant cells in Hodgkin's lymphoma, has led to the prediction that CHD3 may be associated with human prostate cancer (Lemos et al., 2002; Schwad et al., 1982). Interestingly, CHD1 has

been specifically identified as the 5q21 tumor suppressor, playing an important role in the development of human prostate cancer with the deletion of CHD1 in cancer cells leading to an increase in cell invasiveness (Burkhardt et al., 2013; Huang et al., 2011). Further, CHD1 is one of the most frequently deleted genes in prostate cancer patients, to the extent that the deletion has been proposed to be used as a genetic marker for prostate cancer screenings, much like the BRCA1/2 genes may be an indicator for ovarian cancer (Liu et al., 2011; Vijayalakshmi et al., 2016). Understanding more about the CHD proteins can reveal important insights about their associated diseases and conditions.

CHD1 is highly conserved across eukaryotes (Marfella and Imbalzano, 2007). In mice, CHD1 is crucial for the regulation of chromatin and stem cell pluripotency in early embryogenesis (Gaspar-Maia et al., 2009). Drosophila CHD1 has important impacts on fertility and wing development (McDaniel et al., 2008). However, despite previous research illuminating the structure of CHD1, its exact function and mechanisms of action remain unclear. Specifically, understanding CHD1's interaction partners and the mechanism of these interactions requires further study.

Previous research into the binding pattern of *Drosophila* CHD1 on larval polytene chromosomes has demonstrated that CHD1 localizes to sites of interbands, euchromatin, and puffs (sites of swelling along specific sites on polytene chromosomes) associated with high transcriptional activity, suggesting that it functions to alter chromatin to facilitate gene expression (Stokes et al., 1996; Srinivasan, 2005). Interestingly, loss of CHD1 results in an increase in heterochromatin protein HP1 and heterochromatin mark H3K9me2, while altering levels of CHD1 results in defects in the structure of polytene chromosomes (Bugga et al., 2013). These results demonstrate that maintaining CHD1 levels are crucial for chromatin formation and

are supportive of a model of CHD1 function that predicts that it acts counter to heterochromatin by altering chromatin structure (Bugga et al., 2013). This is further supported by evidence that demonstrates that *Drosophila* CHD1 localizes to active regions of polytene chromosomes in a pattern similar to RNA polymerase II, and reminiscent of CHD1's association to RNA polymerase II elongation factors in yeast (Srivasinan et al., 2005; Bugga et al., 2013; Simic et al., 2003).

CHD1 has also been identified as an important component of chromatin (Lusser et al., 2005; Ocampo et al., 2016). Specifically, micrococcal nuclease assays of Drosophila CHD1 demonstrated that CHD1 directs shorter nucleosome spacing compared to ISWI, another ATP-dependent chromatin remodeler (Lusser et al., 2005). Other analyses in *S. cerevisiae* using genome-wide nucleosome sequencing also found that (Ocampo et al., 2016). The study on *S. cerevisiae* found that while ISWI directs for longer spacing and higher-order chromatin folding and condensation, CHD1 directs short spacing, leading to chromatin unfolding and greater transcriptional activity (Ocampo et al., 2016). Though counterintuitive, the shorter nucleosome spacing may enhance transcription by preventing the binding of other proteins (such as Histone H1) that act to repress transcription (Ocampo et al., 2016). Though both CHD1 and ISWI are important for directing nucleosome spacing, the competition between the two remodelers and the differing nucleosome spacing that they direct results in highly dynamic nucleosome structure.

Finally, CHD1 has been shown to interact with histones and histone modifications to direct transcriptional activity. In humans, CHD1 has been shown to bind directly to H3K4, a methylation of Histone H3 that is associated with transcriptional activation (Sims et al., 2005; Chong et al., 2020). This is consistent with research that reveals that CHD1 works as a component of the yeast acetyltransferase complexes, SAGA and SLIK, to recognize and interact

with the methylated lysine 4 mark of histone H3, that is associated with transcriptional activity (Pray-Grant, 2005). In *Drosophila,* CHD1 is required for the deposition of the histone variant H3.3, a key substrate for replication-independent chromatin assembly (Radman-Livaja et al., 2012). In addition, the elimination of CHD1 prevents the incorporation of H3.3 into the male pronucleus and leads to the development of haploid embryos in *Drosophila* (Konev et al., 2011).

Interestingly, a previous study demonstrated that *Drosophila* CHD1 may have revealed a shared regulatory program between CHD1 and Histone H1, another transcription factor that is essential in all metazoans. By analyzing the transcript profiles of CHD1 and H1, they revealed that the proteins share roles in the repression of immune and stress-response related genes (Kavi et al., 2015). Moreover, Vicky Lu, a previous student in the Armstrong Lab observed that the overexpression of CHD1 in *Drosophila* polytene chromosomes resulted in the loss of H1, leading to the proposal that CHD1 works to evict H1 to bind to linker DNA (Vicky Lu, Scripps ’20).

Broadly, CHD1 has been identified as a key modifier of chromatin and has been implicated in a number of biochemical mechanisms. Given the both the highly conserved nature of CHD1 and the implications that alterations to its function can have on gene expression and chromatin formation, understanding its biochemical partners and mechanisms of action are important areas of study.

H1 binding and function

Histone H1, also known as Linker Histone H1, is a crucial component of chromatin structure, present in over 80% of nucleosomes in chromatin (Bustin et al., 2005). H1 exists in multiple isoforms and undergoes many post-translational modifications and is linked it to a variety of developmental functions. H1 is also able to demonstrate distinct species, tissue, and developmental specificity (Bustin et al., 2005).

H1 binds with the linker DNA, interacting with ~20 base pairs at the entry/exit sites of the nucleosome, not inclusive of the 147 nucleotide pairs of the nucleosome core DNA. (Happel and Doenecke, 2009). The resultant particle, consisting of about 167 nucleotide pairs of DNA, the core histone octamer and one molecule of H1, is known as the chromatosome (Simpson, 1980). Currently, H1 is predicted to function within a network of chromatin binding proteins to modulates the activity of nucleosome remodeling complexes (Bustin et al., 2005). This network notably includes nuclear proteins that have been shown to displace H1, resulting in transcriptional activation, such as the glucocorticoid receptor, upstream binding Factor (UBF), and liver-enriched transcription factor (HNF-3) (Zlatanova et al., 2000). Broadly, genetic knockout experiments, such as those in in *T. thermophila*, *S. cerevisiae*, and in *M. musculus*, established roles for H1 in chromatin condensation and nucleosome stabilization (Shen et al., 1996; Patterton et al., 1998; Fan et al., 2003). The loss of *Drosophila* H1 function leads to defects in gene expression, chromosome decondensation, genomic instability, and lethality (Siriaco et al., 2015). Perhaps unexpectedly, given the role that plays in maintaining chromatin structure, H1 binding is highly dynamic, continuously exchanging between chromatin sites in a "stop and go" process (Izzo et al., 2008). H1 variants may display differential DNA binding affinities due to variations in their C-terminal tails, interactions with co-factors that recruit H1 to

certain locations in the DNA, and complex interactions with other proteins in its network of chromatin binding proteins (Catez et al., 2006).

Recently, H1 has been emerging as a point of interest due to its frequent mutation in cancer cells. Exosome sequencing and single nucleotide polymorphism array profiling of follicular lymphoma (the most common non-Hodgkin lymphoma in the Western world) revealed mutations in four Histone H1 variants (*HISTH1 B-E)* to be recurrently mutated in cancer cells (Li et al, 2014). Moreover, Histone variant H1.5 has been strongly identified as a potential biomarker for prostate cancer in humans, with an immunohistochemical study demonstrating strong nuclear reactivity in 93% in all prostate adenocarcinomas, compared to only 9% of benign samples (Khachaturov, et al 2014). Meanwhile, H1 depletion in *D. melanogaster* and *M. musculus* was also shown to cause lethality in early development (Fan et al, 2003; Lu et al, 2009). Taken together, this evidence highlights an increasing necessity to understand H1 function.

Currently, H1 is known to play an important role in transcription regulation, though its exact function is unclear. *In vitro* studies have generally led to the assumption that H1 functions as a global transcription repressor by promoting more condensed chromatin structure (Zlatanova, 1990), though there is more recent, growing evidence to support its implications in other cellular functions. Notably, global H1 deletions in chicken embryos resulted in a downregulation of transcription of genes H1-deficient cells, indicating that H1 may be responsible for some amount of transcriptional activation, though some transcripts were upregulated as well. Chicken cells lacking H1 also demonstrated a decrease in nuclear spacing and an overall increase in nuclear volume (Hashimoto et al., 2010). Similarly, depletions of individual H1 variants in human cell lines indicated tendencies for the activation of gene expression (Sancho et al., 2008).

Moreover, a recent study concluded that H1 is implicated in both the repression of some euchromatic genes, as well as the activation of some heterochromatic genes, despite earlier evidence that characterized H1 as a transcription repressor (Lu et al., 2009). This study also demonstrated that depletion of H1 to ~20% of the level in wild-type larvae results in lethality, and showed that H1 is necessary for *Drosophila* development, the establishment of pericentric heterochromatin, and the alignment of sister chromatids (Lu et al, 2009). This is supplemented by other analyses that implicate H1 in having an essential role in chromosome segregation in *Xenopus laevis* (Maresca et al., 2005) and as a repressor of homologous recombination in yeast (Kalashnikova et al, 2016). Taken together, this evidence points to a wide variety of roles that H1 may have in a variety of cellular processes and makes it difficult to classify H1 solely as a transcriptional activator or repressor.

There is increasing documentation of interactions between H1 (and its variants) and a variety of proteins involved in transcription regulation and chromatin remodeling (Kalashikova et al, 2016; Zlatanova et al, 2000). However, despite the existing body of research, H1 function--particularly its interactions with functional partners--remain largely unknown. H1 function can be difficult to study, especially given the multitude of H1 variants and the number of factors that it is implicated in. *Drosophila melanogaster* offers an excellent model organism for this investigation, as *Drosophila* only possess one variation of H1, compared to other metazoans (Bayona-Feliu, 2016).

Notably, a screen for enhancers and suppressors of lethality induced by loss of H1 identified a strong interaction between CHD1 and H1 (Kavi et al., 2013). The study demonstrated that the reduced viability resulting from loss of H1 was altered, related to the changed levels of CHD1 expression. Specifically, reduced expression of *CHD1* resulted in an

enhancement of the semi-lethal phenotype caused by *H1* knockdown (Kavi et al., 2013). Moreover, analysis of H1 and CHD1's transcription profiles revealed that the proteins share roles in the repression of immune-related and stress response-related genes, suggesting that CHD1 and H1 may share a common transcriptional regulatory program (Kavi et al., 2013). These results indicate that H1 and CHD1 may work in conjunction to regulate transcription.

However, other studies found that CHD1 and H1 may act in opposition to each other in terms of nucleosome assembly. A study of CHD1 in *S. cerevisiae* was demonstrated that CHD1 works to oppose ISWI to direct short nucleosome spacing, resulting in the eviction of H1 (Ocampo et al., 2016). Similarly, an *in vitro* study found that *Drosophila* CHD1 was not able to assemble chromatin containing H1, indicating that CHD1 and H1 may be incompatible (Lusser et al., 2005). Mentioned above, Vicky Lu, a student in the Armstrong lab (Scripps '20) completed analyses using confocal microscopy of live nuclei that demonstrated a competitive interaction between CHD1 and H1. This research led to the proposal of a model in which CHD1 works antagonistically to H1 to prevent H1 binding to linker DNA (Vicky Lu, Scripps '20). In this working model, CHD1 binding evicts H1 deposition, leading to greater transcriptional activity and shorter nucleosome spacing (Vicky Lu, Scripps '20).

H1 has been implicated in a variety of cellular processes and understanding the protein-protein interactions that it is involved in may be key to understanding its function. CHD1 has been identified as one of H1's potential functional partners, with the proteins sharing roles in the repression of transcription (Kavi et al., 2013). However, other work, including research produced by the Armstrong lab, provides evidence for a competitive interaction between the two proteins. The exact nature of the interaction between CHD1 and H1 stands to be further clarified.

CHD1 overexpression in the wing as a basis for a genetic interaction assay

Drosophila melanogaster is an ideal model organism for studying CHD1, as alterations to CHD1 levels produce robust phenotypes, and because CHD1 is not necessary for *Drosophila* viability. Previous students in the Armstrong lab identified that the overexpression of CHD1 leads to wing vein defects, including wing vein branching and blistering, compared to wild-type wings (Parimal Deodhar, Scripps '07; Sharon Kim, Scripps '13; Figure 1).

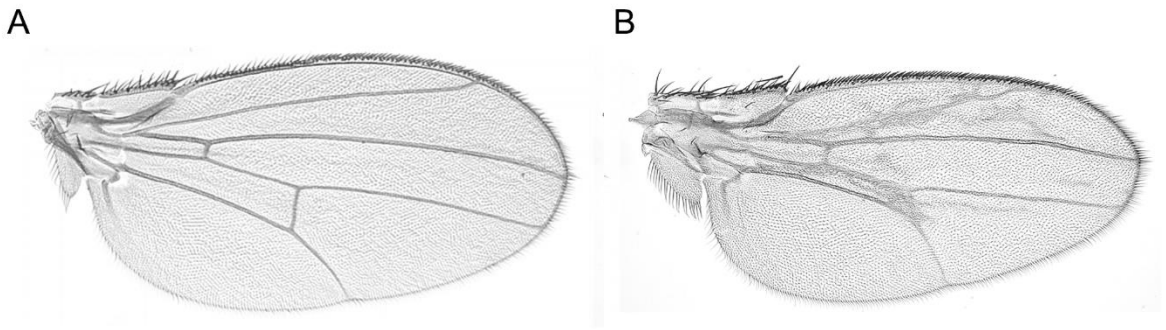

Figure 1. Wing defects caused by overexpression of CHD1. Compared to the wild-type (Oregon R) wing (left), CHD1 overexpression (*w; P[w+ ,UAS-chd1+]113/ P[w+ ,69B-GAL4](R2))* causes wing vein defects (Figure from Sharon Kim, Scripps '13).

The overexpression of CHD1 in the wing is accomplished by using the GAL4/UAS system, a two-part inducible system that makes use of the yeast transcription activator GAL4 and the transcription enhancer/GAL4 binding site, Upstream Activating Sequence (UAS) (Brad and Perrimon, 1993; Figure 2). Typically, UAS fly lines carry the UAS element attached to a gene of interest. These UAS flies are crossed to a fly line that express GAL4 in specific tissues. This system was selected because only flies containing both the UAS and the GAL4 elements will express the gene of interest. For the work in my thesis, I used the *69B-GAL4* driver to drive gene expression in larval imaginal wing discs.

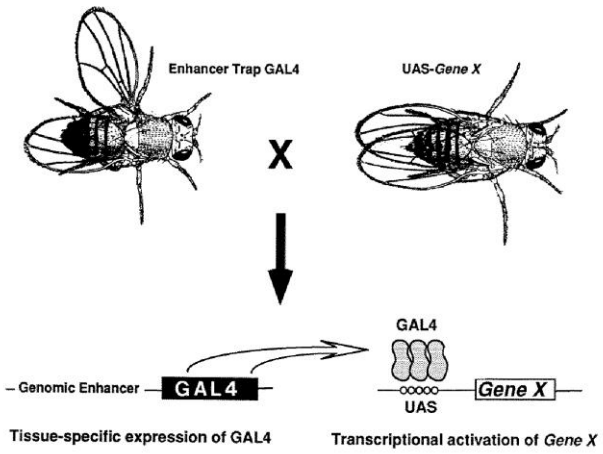

Figure 2. GAL4/UAS-directed gene expression in *Drosophila*. A fly expressing the tissue-specific GAL4 driver is crossed to a fly containing the UAS element. When GAL4 binds to UAS, Gene X is overexpressed. Figure from Brand and Perrimon, 1993.

To facilitate the use of this genetic system, Ivy McDaniel (Scripps ’08) created a recombinant stock that carried both the *UAS-chd1* and the *69B-GAL4* transgenes on one chromosome. Eugenie Hong (Scripps ’11) used the wing vein defects that resulted from the overexpression of CHD1 to develop a sensitized genetic screen to identify factors that functionally interact with CHD1. The Armstrong lab subsequently incorporated a balancer chromosome expressing the GAL80 repressor to repress GAL4/UAS activity in the parent stocks to reduce the chance for selection of second site modifiers. When the recombinant stock was crossed with another stock, thus losing the chromosome with GAL80, CHD1 was overexpressed in the progeny.

As mentioned, Eugenie Hong (Scripps, ’11) developed a sensitized genetic screen for factors that interact with CHD1. The screen took advantage of the powerful wing vein defect phenotype that CHD1 overexpression produced in order to identify other genes whose alteration would either enhance or suppress that phenotype. Partners were identified by using RNA-

interference, or RNAi, to knock down expression of the gene that is suspected to interact with CHD1 (Figure 3). If the protein product of the knocked down gene interacts with CHD1, then we predicted that the wing phenotype associated with CHD1 would be altered in some way.

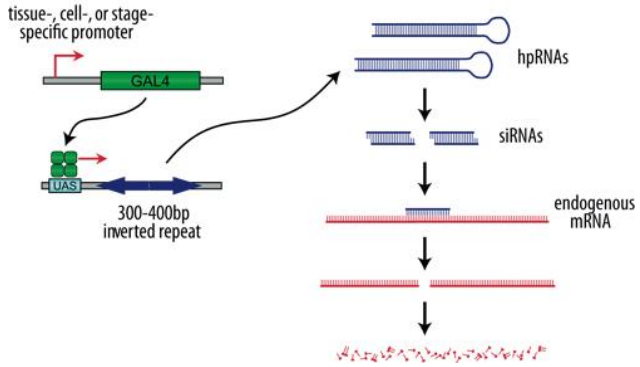

Figure 3. RNAi-mediated knockdown of a gene using GAL4/UAS. When GAL4 binds to the UAS element, it expresses a hairpin RNA that targets the gene of interest. The mRNA of that gene is degraded, and the gene is not expressed.

RNAi-based technologies have been widely used to knock down gene expression and identify novel genes and genetic interactions. The introduction of small, double-stranded RNA that results in sequence-specific silencing (Fire et al., 1998) and can be induced by way of the GAL4/UAS system to observe the knockdown of genes in a tissue-specific manner (Perkins et al., 2015). My work specifically used the VALIUM20 (**V**ermilion-**A**ttB-**L**oxp-**I**ntron-**U**AS-**M**CS) vector for sequence-specific silencing of certain genes (Perkins et al., 2015). VALIUM20 offers a very strong knockdown of genes of interest, allowing us to evaluate the consequences of loss of target proteins on the CHD1 over-expression wing-based genetic assay system (Ni et al., 2010).

Exploring the GAL4/UAS system

An ideal negative control for this experiment is a cross in which CHD1 is overexpressed while RNAi is used to knock down a gene that does not affect CHD1 activity. A negative control cross knocking down *mCherry* while overexpressing CHD1 was specifically selected because *mCherry* is not found in *Drosophila.* Thus, RNAi directed against *mCherry* mRNA was not expected to affect *CHD1* activity and should not have had an effect on the wing vein defects that result from over-expression of CHD1. To our initial surprise, overexpressing *CHD1* while expressing mRNAi directed against *mCherry* mRNA resulted in the suppression of the wing vein defects (Figure 4; Sharon Kim, Scripps '13).

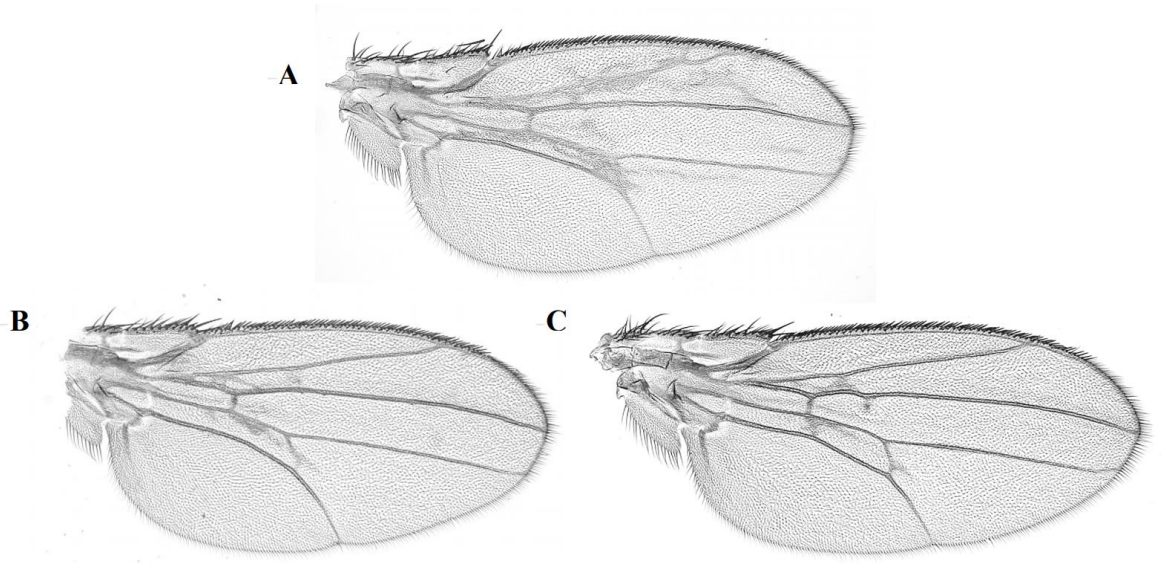

Figure 4. Wing vein defects are less severe in flies using RNAi to knockdown gene expression. (A) Overexpression of *CHD1* leads to severe wing vein branching (*w; +; P[w+ ,UAS-chd1+]113, P[w+ ,69B-GAL4]*). (B) Overexpression of *CHD1* and expression of *mCherry* RNAi leads to a less severe, suppressed phenotype (*w; +; P[w+ ,UAS-chd1+]113, P[w+ ,69B-GAL4](R2)/RNAi-mCherry).* (C) Overexpression of *CHD1* and knockdown of *luciferase* also leads to a suppressed phenotype (*w; +; P[w+ ,UAS-chd1+]113, P[w+ ,69B-GAL4](R2)/RNAi-luciferase*) (Sharon Kim, Scripps '13).

Sharon Kim speculated that the suppression of the wing phenotype is because the RNAi line itself uses a UAS element. Sharon concluded that the *UAS-RNAi* element in conjunction with *UAS-CHD1* could have the unwanted effect of titrating away GAL4 from *UAS-CHD1*, thus affecting the strength of the wing defect phenotype (Figure 5A). In my investigation, I hypothesized that, if GAL4 is limiting in our flies overexpressing *CHD1* and knocking down *mCherry*, then the introduction of more GAL4 will result in more severe wing defect phenotypes (Figure 5B).

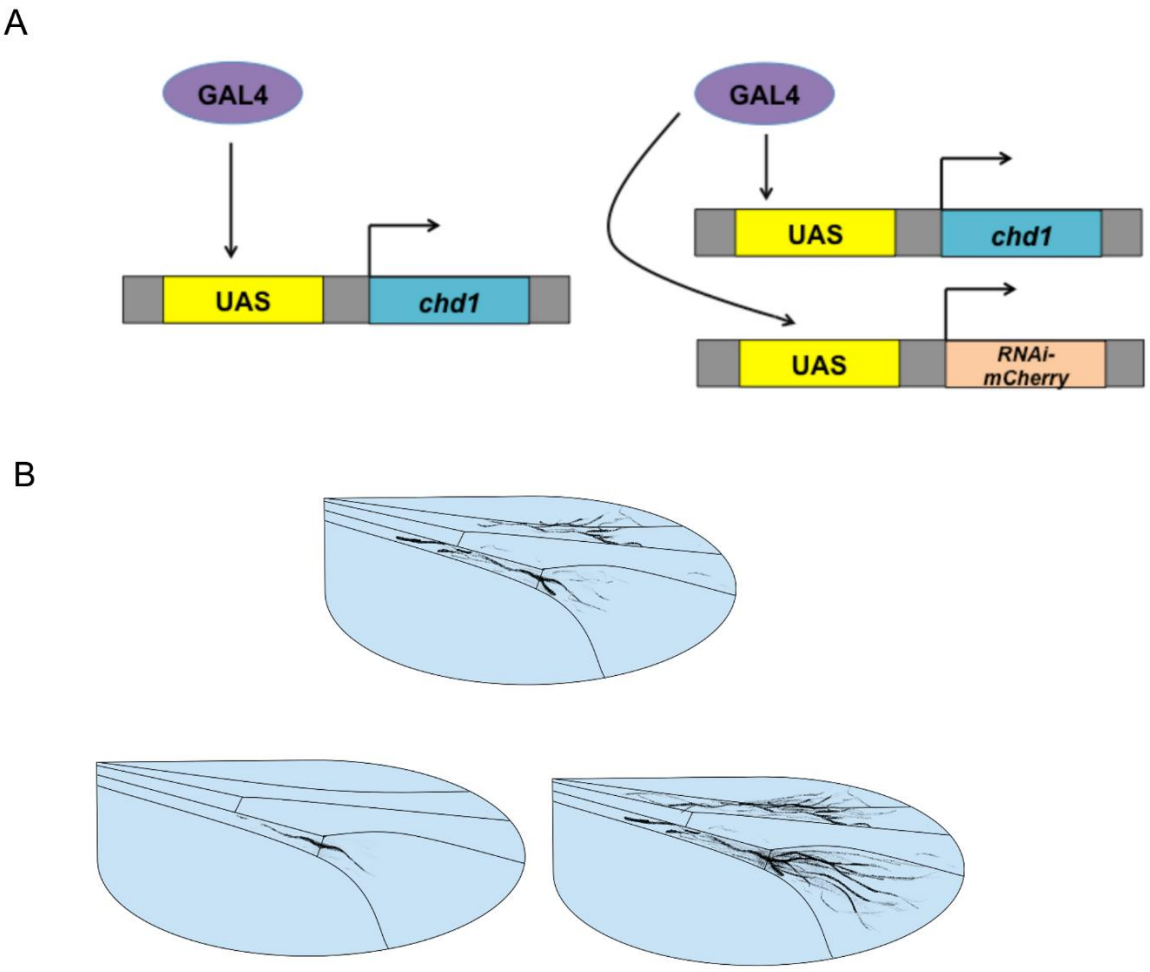

Figure 5. Proposed titration away of GAL4 by *RNAi mCherry*. (A) On the left, GAL4 is used for the expression of the *CHD1* protein. On the right, GAL4 is used for both the expression of *CHD1* and the knockdown of *mCherry* through use of RNAi. The extra *UAS* element could titrate away GAL4 originally used to overexpress *CHD1*. Figure from Sharon Kim's thesis (Scripps '13). (B) Introducing more GAL4 to the system by crossing the recombinant stock containing *GAL4* and *UAS* elements to flies containing an additional copy of the *GAL4* driver in order to test if GAL4 levels are limiting flies overexpressing *CHD1* ($P[w^+, UAS-chd1^+]113, P[w^+, 69B-GAL4](R2)/P\{w[^+mW.hs] = GawB\}69B-Gal4$). Top: If GAL4 is not limiting, introducing more GAL4 will not cause a change in the severity of wing vein defects. Left: If GAL4 is limiting, introducing more GAL4 will lead to a suppression in the severity of the wing vein phenotype. Right: If there is too much GAL4, introducing more GAL4 will lead to an enhancement of the vein defect phenotype.

Investigating a working model of CHD1 and H1 interaction

Vicky Lu, a previous student in the Armstrong lab, proposed a working model for CHD1 and H1 interaction. She used *in vivo* analyses of polytene chromosomes to visualize H1 signal in

response to varied CHD1 levels and found that CHD1 overexpression led to a decrease in levels of H1 (Vicky Lu, Scripps ’20). She specifically proposed that CHD1 physically competes with H1, while facilitating the deposition of H3.3, which acts to further prevent H1 binding. Her model suggests that CHD1 directs shorter nucleosome spacing, excluding the deposition of H1 (Vicky Lu, Scripps ’20).

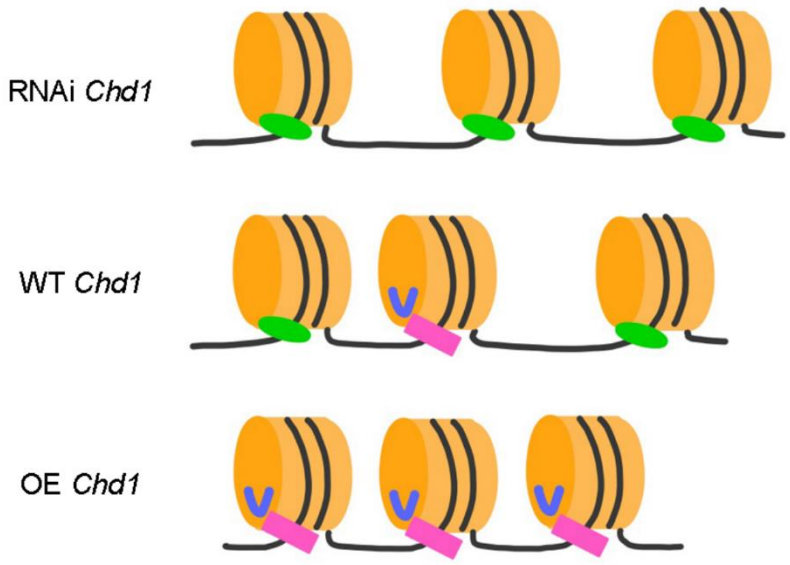

Figure 6. H1 and CHD1 interact competitively to mediate histone spacing. Model demonstrating how RNAi-CHD1, wild type CHD1, and CHD1 overexpression may alter deposition of H1 and histone variant H3.3, thus affecting nucleosome spacing. Nucleosomes are represented by orange cylinders, DNA is represented by black lines, H3.3 is represented by purple marks on the nucleosome core, CHD1 is represented by green ovals, and H1 is represented by pink rectangles (Vicky Lu, Scripps ’20).

My experiment builds on the genetic screen that had been developed by other students, taking a genetic approach to studying test this working model. As mentioned, the I assumed that if the protein product of the knocked down gene interacts with CHD1, then we would expect that the wing phenotype associated with CHD1 would be altered in some way. This same reasoning was applied to this investigation for clarifying the interaction between CHD1 and H1.

Specifically, if H1 works together with CHD1, then the wing defect phenotype caused by over-expression of *CHD1*

will be suppressed following the loss of H1. On the other hand, if H1 works antagonistically with CHD1, the wing defect phenotype caused by over-expression of *CHD1* would be enhanced by loss of H1 (Figure 6).

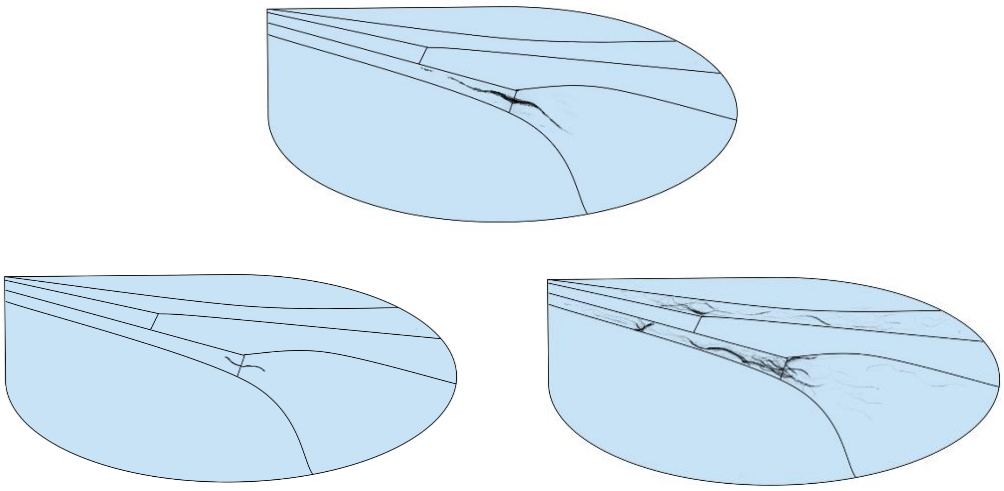

Figure 7. Using the novel genetic assay. Top: If H1 and CHD1 do not functionally interact, there would be no change in the severity of wing vein defects caused by over-expression of *CHD1*. Left: If H1 works together with CHD1, there will be a suppressed wing defect phenotype as result of loss of H1. Right: If H1 works antagonistically with CHD1, there will be an enhanced wing defect phenotype in flies over-expressing *CHD1* with reduced *H1*.

Materials and Methods

Drosophila stocks and crosses

Stocks were raised at 24 °C on a standard medium containing cornmeal, molasses, yeast, agar, Tegosept, and propionic acid. Fly food was stored at 4 °C before use. Stocks were flipped every 3 weeks. Flies were anaesthetized using carbon dioxide, and flies with the desired phenotypes for crosses were selected. Fly stock genotypes and their respective sources are shown below (Table 1). Crosses were propagated using 5-6 male flies and 5-6 female flies. Virgin female flies were specifically selected to propagate crosses. After 5-7 days, crosses were flipped to new vials to prevent overcrowding. Crosses were performed at both 24 °C and 29 °C unless otherwise indicated. Right wings of male flies were removed at day 18 for flies raised at 24 °C and day 14 for flies raised at 29 °C. Wings stored in isopropanol for 2-3 days before mounting.

Table 1. List of stocks and their respective sources.

Stock	**Source**
$w; \frac{P[w^+, UAS-chd1^+]113, P[w^+, 69B-GAL4](R2)}{TM3, Sb, Tub-GAL80, w^+}$	Ivy McDaniel
$w^+; P\{w[^+mW.hs] = GawB\}69B\text{-}Gal4$	Bloomington Stock Center
$y' sc^* v'; +; P\{VALIUM20-mCherry\} attP2 (RNAi-mCherry)$	Bloomington Stock Center
$y' sc^* v'; +; P\{y^{+t7.7} v^{+t1.7} = TRiP.HMS005863\} attP2 (RNAi-Ino80)$	Bloomington Stock Center
$y' v'; P\{y^{+t7.7} v^{+t1.7} = TRiP.JF01557\} attP2 (RNAi-trxA)$	Harvard Medical School TRiP
$y' v'; P\{y^{+t7.7} v^{+t1.7} = TRiP.JF01582\} attP2 (RNAi-ISWIA)$	Harvard Medical School TRiP
$y, w; +; UAS-H1RNAi8-4$	Giorgia Siriaco
$y, w; +; UAS-H1RNAi10-3$	Giorgia Siriaco

Table 2. Genotypes of parent cross and progeny for baseline controls for wing vein defects caused by *CHD1* Overexpression

	Negative Control
Parent:	♀ $\frac{y'sc^*v'}{y'sc^*v'};\frac{+}{+};\frac{P\{VALIUM20-mCherry\}attP2(RNAi-mCherry)}{P\{VALIUM20-mCherry\}attP2(RNAi-mCherry)};\frac{+}{+}$ **X** ♂ $\frac{w}{\rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{TM3,Sb,Tub-GAL80,w^+};\frac{+}{+};\frac{+}{+}$
F1:	$\frac{w}{y'sc^*v' \text{ or } \rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{+};\frac{P\{VALIUM20-mCherry\}attP2(RNAi-mCherry)}{+};\frac{+}{+}$
	Positive Control
Parent:	♀ $\frac{y'sc^*v'}{y'sc^*v'};\frac{+}{+};\frac{P\{y^{+t7.7}v^{+t1.7}=TRiP.HMS005863\}attP2(RNAi-Ino80)}{P\{y^{+t7.7}v^{+t1.7}=TRiP.HMS005863\}attP2(RNAi-Ino80};\frac{+}{+}$ **X** ♂ $\frac{w}{\rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{TM3,Sb,Tub-GAL80,w^+};\frac{+}{+};\frac{+}{+}$
F1:	$\frac{w}{y'sc^*v' \text{ or } \rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{+};\frac{P\{y^{+t7.7}v^{+t1.7}=TRiP.HMS005863\}attP2(RNAi-Ino80)}{+};\frac{+}{+}$

Table 3. Genotypes of parent cross and progeny confirming GAL4/UAS system function

	ISWI and trx crosses
Parent:	♀ $\frac{y'v'}{y'v'};\frac{+}{+};\frac{P\{y^{+t7.7}v^{+t1.7}=TRiP.JF01582\}attP2(RNAi-ISWIA)}{P\{y^{+t7.7}v^{+t1.7}=TRiP.JF01582\}attP2(RNAi-ISWIA)};\frac{+}{+}$ **X** ♂ $\frac{w}{\rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{TM3,Sb,Tub-GAL80,w^+};\frac{+}{+};\frac{+}{+}$
F1:	$\frac{y'v'}{w \text{ or } \rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{+};\frac{P\{y^{+t7.7}v^{+t1.7}=TRiP.JF01582\}attP2(RNAi-ISWIA)}{+};\frac{+}{+}$
	AND
Parent:	♀ $\frac{y'v'}{y'v'};\frac{+}{+};\frac{P\{y^{+t7.7}v^{+t1.7}=TRiP.JF01557\}attP2(RNAi-trxA)}{P\{y^{+t7.7}v^{+t1.7}=TRiP.JF01557\}attP2(RNAi-trxA)};\frac{+}{+}$ **X** ♂ $\frac{w}{\rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{TM3,Sb,Tub-GAL80,w^+};\frac{+}{+};\frac{+}{+}$
F1:	$\frac{y'v'}{w \text{ or } \rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{+};\frac{P\{y^{+t7.7}v^{+t1.7}=TRiP.JF01557\}attP2(RNAi-trxA)}{+};\frac{+}{+}$
	Exploring Saturation of UAS Binding Sites
Parent:	♀ $\frac{w}{w};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{TM3,Sb,Tub-GAL80,w^+};\frac{+}{+};\frac{+}{+}$ **X** ♂ $\frac{w^+}{\rightarrow};\frac{P\{w[^+mW.hs]=GawB\}69B-Gal4}{P\{w[^+mW.hs]=GawB\}69B-Gal4};\frac{+}{+};\frac{+}{+}$
F1:	$\frac{w}{w^+ \text{ or } \rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{P\{w[^+mW.hs]=GawB\}69B-Gal4};\frac{+}{+};\frac{+}{+}$

Table 4. Genotypes of parent cross and progeny with *CHD1* overexpression and simultaneous *H1* knockdown and their respective control

	***H1* Knockdown Without *Chd1* Overexpression**
Parent:	♀ $\frac{y,w}{y,w};\frac{+}{+};\frac{UAS-H1RNAi8-4}{UAS-H1RNAi8-4};\frac{+}{+}$ **X** ♂ $\frac{w^+}{\rightarrow};\frac{P\{w[^+mW.hs]=GawB\}69B-Gal4}{P\{w[^+mW.hs]=GawB\}69B-Gal4};\frac{+}{+};\frac{+}{+}$
F1:	$\frac{y,w}{w^+ \text{ or } \rightarrow};\frac{P\{w[^+mW.hs]=GawB\}69B-Gal4}{+};\frac{UAS-H1RNAi8-4}{+};\frac{+}{+}$
	AND
Parent:	♀ $\frac{y,w}{y,w};\frac{+}{+};\frac{UAS-H1RNAi10-3}{UAS-H1RNAi10-3};\frac{+}{+}$ **X** ♂ $\frac{w^+}{\rightarrow};\frac{P\{w[^+mW.hs]=GawB\}69B-Gal4}{P\{w[^+mW.hs]=GawB\}69B-Gal4};\frac{+}{+};\frac{+}{+}$
F1:	$\frac{y,w}{w^+ \text{ or } \rightarrow};\frac{P\{w[^+mW.hs]=GawB\}69B-Gal4}{+};\frac{UAS-H1RNAi10-3}{+};\frac{+}{+}$
	***Chd1* Overexpression with Simultaneous *H1* Knockdown**
Parent:	♀ $\frac{y,w}{y,w};\frac{+}{+};\frac{UAS-H1RNAi8-4}{UAS-H1RNAi8-4};\frac{+}{+}$ **X** ♂ $\frac{w}{\rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{TM3,Sb,Tub-GAL80,w^+};\frac{+}{+};\frac{+}{+}$
F1:	$\frac{w}{y,w \text{ or } \rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{+};\frac{UAS-H1RNAi8-4}{+};\frac{+}{+}$
	AND
Parent:	♀ $\frac{y,w}{y,w};\frac{+}{+};\frac{UAS-H1RNAi10-3}{UAS-H1RNAi10-3};\frac{+}{+}$ **X** ♂ $\frac{w}{\rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{TM3,Sb,Tub-GAL80,w^+};\frac{+}{+};\frac{+}{+}$
F1:	$\frac{w}{y,w \text{ or } \rightarrow};\frac{P[w^+,UAS-chd1^+]113,P[w^+,69B-GAL4](R2)}{+};\frac{UAS-H1RNAi10-3}{+};\frac{+}{+}$

Wing Mounting

Using forceps, remove right wings of male flies and store in 600 µL isopropanol for at least 48 hours. Pipette 1-3 wings onto a slide and position using forceps. If the wings are placed more closely together or if there is only one wing, place a small drop of Canada Balsam (Sigma Aldrich) in the center of the coverslip on the center of a glass coverslip using a P100 pipette tip. If the wings are spread father apart on the slide, or if there are multiple wings, spread the Canada Balsam in a thin line in the center of the coverslip so that there is enough to encapsulate all the wings. While spreading the Canada Balsam, avoid lifting the pipette tip off the slide to prevent creating bubbles. Canada Balsam can become more viscous if left for too long or exposed to too much heat, causing bubbles on the slide. Order fresh if necessary.

Working quickly to avoid letting the isopropanol on the slide drying, place the coverslip on top of the wings. If the isopropanol dried before the coverslip is placed, re-wet the slip with 1-2 drops of isopropanol and wait roughly 30 seconds before placing the coverslip. If necessary, reposition the wings on the slide using forceps. Expect that the wings may move when the coverslip is placed. View the wings under a Zeiss light microscope to ensure that wings are not folded. Small adjustments may be made to unfold wings, but special attention must be made so that wings do not tear.

Place a bolt on top of the coverslip as a weight and incubate the slides overnight at 55˚C. The wings were viewed on a Leica DM 4000 B LED microscope using a 5 x objective. Images within each figure were processed identically using Photoshop Adobe CS5.

Wing Scoring

To determine whether H1 knockdown and simultaneous CHD1 overexpression resulted in significant changes in in wing vein defects, a scoring system was used, in which wings were divided into cells. The scoring system was adapted from Sharon Kim's previous work (Sharon Kim, Scripps '13). Wing cells are divided by wild-type wing veins into 5 regions. Wings were scored on a scale from 0-5 depending on the presence of wing vein defects in these cells (Figure 5). Scoring was performed using a Zeiss light microscope before wings were mounted.

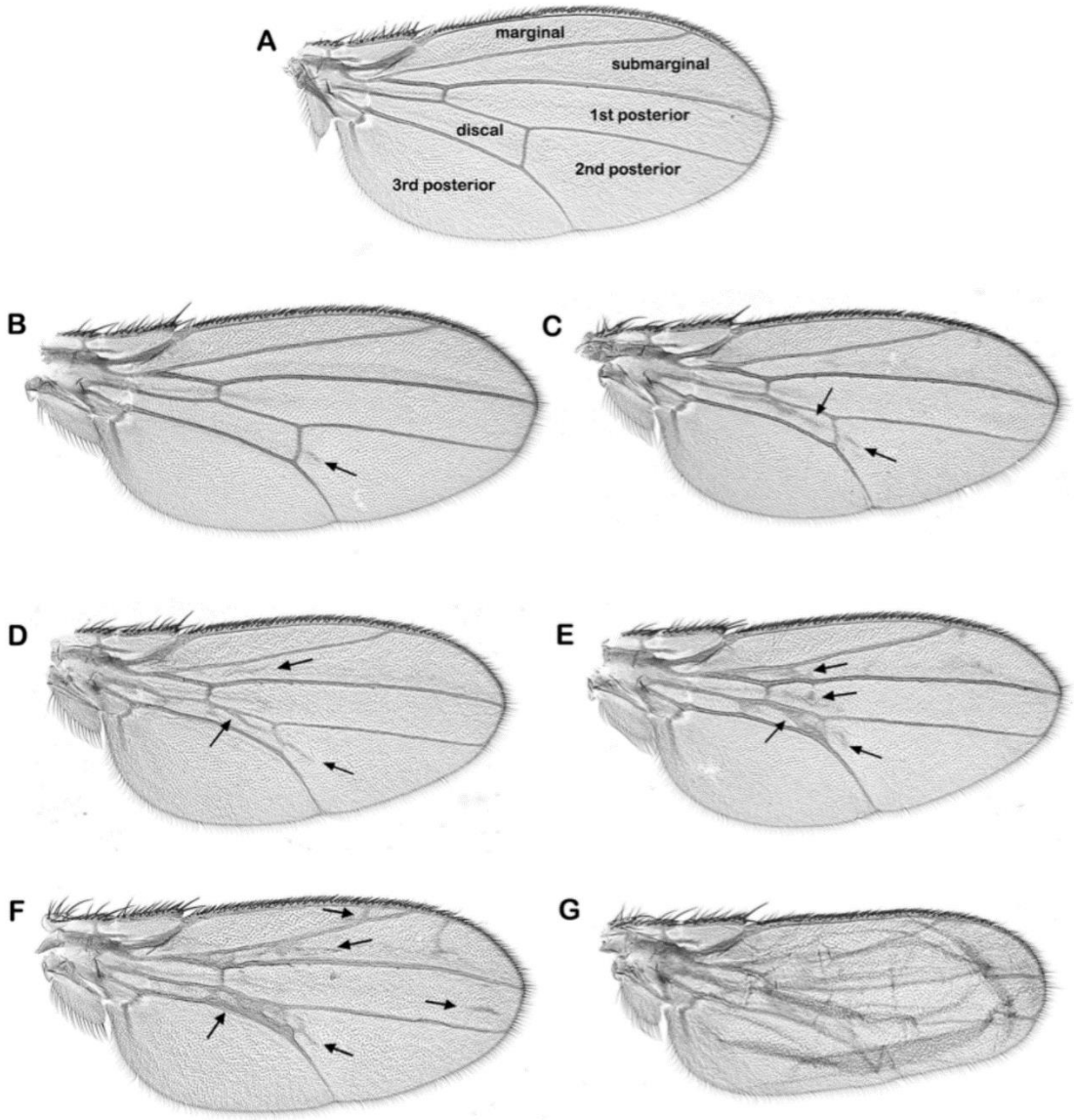

Figure 6. (A) Wings were divided into cells (marginal, submarginal, discal, first posterior, second posterior, third posterior), based on wild-type wing veins. Wild-type wings received a score of 0. (B) Wing with one defect in the second posterior cell, receiving a score of 1. (C) Wing with defects in the discal and second posterior cells, receiving a score of 2. (D) Wing with defects in the submarginal, discal, and second posterior cells, receiving a score of 3. (E) Wing with defects in the submarginal, discal, first posterior, and second posterior cells, receiving a score of 4. (F) Wing with defects in the marginal, submarginal, discal, first posterior, and second posterior cells, receiving a score of 4. (G) An example of a wing with a blistered phenotype, the most extreme wing defect phenotype. Blistered wings were scored B (Sharon Kim, Scripps '13)

Results

I used a genetic assay that makes use of the wing vein defect phenotypes caused by the overexpression of *CHD1* to investigate the interaction between CHD1 and H1. More specifically, I investigated Vicky Lu's working model of CHD1 and H1 interactions in which she proposes that CHD1 and H1 competitively bind to linker DNA to direct nucleosome spacing. I performed an additional test to investigate the limits of the GAL4/UAS system, as it is used in both the overexpression of *CHD1* and the knockdown of *H1*.

Much of this work was completed between Spring 2018 and Spring 2020 as part of ongoing research in contribution to the Armstrong Lab and in preparation for Senior Thesis work. However, due to Stay-at-Home Orders that were announced mid-March 2020, I was not allowed to return to campus to complete certain experiments. However, I am presenting the work that I have completed, in addition to a comprehensive literature review, in addition to directions for further research building off of these experiments are presented here.

Qualitative assessment of wing phenotypes resulting from overexpression of *CHD1*

Sharon Kim developed a quantitative scoring system to identify interactions that enhanced the wing defect phenotype (Sharon Kim, Scripps '13). This scoring system was able to differentiate between wing vein defect severity if wing vein branching was present in multiple cells but did not account for significant changes in wing vein defect phenotypes if they occurred within the same cell. In my crosses, I found that wing vein defects consistently occurred within the same cell. The scoring system was therefore not able to account for the differences in wing vein defect severity encountered in this study. As such, wings were only assessed qualitatively, rather than quantitatively.

Evaluation of wing-based CHD1 genetic assay system

For my genetic assay, it was necessary to establish baseline controls that incorporate GAL4/UAS-driven overexpression of *CHD1* with the simultaneous knockdown of a target gene by RNAi. As mentioned above, this was partly accomplished by crossing flies that contained both the *UAS-CHD1* and the *69B-GAL4* transgenes with flies that knocked down the non-Drosophila gene *mCherry*. I observed and collected the right wings of male flies serve as my negative control for my analysis (Figure 8A; Table 5). This was to ensure that any phenotypes seen are due to an interaction between *CHD1* and the target gene, rather than a complication arising from using the GAL4/UAS system with RNAi. Fly wings are shown below for the results of this cross performed at 24 ˚C. Progeny in which *CHD1* is overexpressed while *mCherry* is being knocked down that were raised at 29 ˚C showed slightly enhanced wing vein defects, relative to the same cross at 24 ˚C. Only one male fly was produced from the cross in which *nCherry* was knocked down with *CHD1* overexpression at 29 ˚C. Recorded data includes observations from male and female flies (Table 5).

Sharon Kim identified a functional interaction between CHD1 and INO80 using a similar genetic assay (Sharon Kim, ’13). Like CHD1, INO80 is an ATP-dependent chromatin remodeler that was shown to direct nucleosome spacing, as well as being implicated in transcription and DNA replication (Udgugama et al., 2011). Previously, when *INO80* was knocked down, the wing defect phenotype caused by *CHD1* overexpression was strongly enhanced (Sharon Kim, ’13). As such, wings with simultaneous *CHD1* overexpression and RNAi-mediated knockdown of *INO80* were observed for positive controls of this experiment. Contrary to previous findings, wing defects phenotypes in these flies were not remarkably different than the negative controls at 24 ˚C. Surprisingly, this cross produced far fewer male flies than female flies (Figure 8B; Table 5). I

could only recover one female fly when this cross was performed at 29 ˚C. Wings of the female fly were blistered, indicating that defects were significantly enhanced (Table 5).

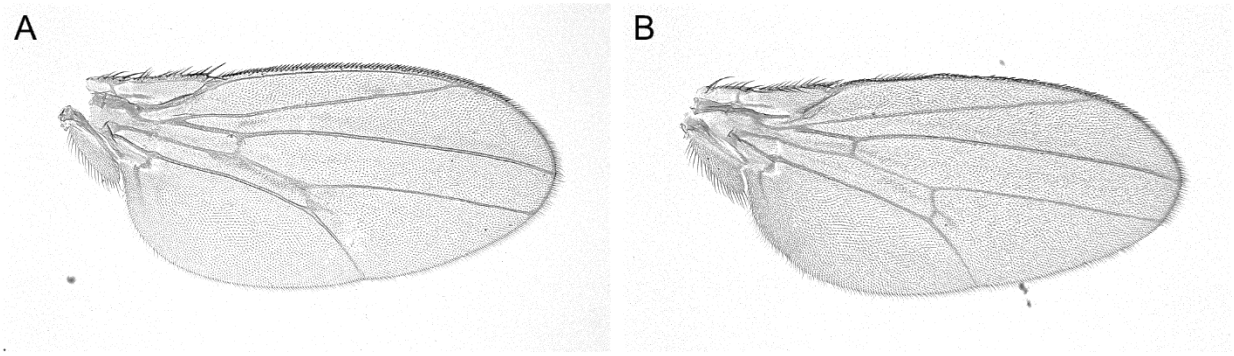

Figure 7. *INO80* knockdown does not enhance wing vein defects caused by overexpression of *CHD1* at 24 ˚C. (A) Overexpression of *CHD1* and knockdown of *mCherry* leads to wing defect phenotypes at 24 ˚C *(w; P[w+, UAS-chd1+]113, P[w+,69B-GAL4](R2)/RNAi-mCherry)*. (B) Overexpression of *CHD1* and knockdown of *INO80* does not lead to more severe wing vein defects at 24 ˚C *(w; P[w+, UAS-chd1+]113; P{y+t7.7v+t1.7=TRiP.HMS005863}attP2(RNAi-Ino80).*

Understanding the behavior of the GAL4/UAS system

To confirm that the GAL4/UAS system behaves in the expected manner, I performed crosses in which the overexpression of *CHD1* with simultaneous targeted knockdowns were previously found to be lethal, specifically *trx* and *ISWI* (Sharon Kim, ’13). *Trithorax*, or *trx*, is a part of the trithorax group of proteins. TRX proteins are required for maintaining the active state of genes by antagonizing Polycomb proteins (Geisler and Paro, 2015). Previous research into shared partners suggests that CHD1 and TRX may act in the same pathway (Srinivasan et al., 2005; Srinivasan et al., 2008). *ISWI*, or *Drosophila* imitation, is an ATP-dependent remodeler that plays roles in cell viability and gene expression that has been shown to compete with CHD1 to direct nucleosome spacing (Corona et al., 1999; Ocampo et al., 2016).

In previous studies, knockdown of both *ISWI* and *trx* using VALIUM20 RNAi lines resulted in lethality at 24 ˚C. VALIUM1 RNAi lines showed that *ISWI* knockdown suppressed

the wing defect phenotype, while *trx* knockdown slightly enhanced the phenotype (Sharon Kim, ’13). Results of this experiment are not consistent with previous findings. Knockdown of *ISWI* and *trx* did not result in lethality at 24 ˚C or 29 ˚C and all progeny had wild-type wings, using the same VALIUM RNAi lines. I did not record fly counts or the sex of flies. Given that I was not able to return to the lab to follow-up these results, I am not able to interpret these results. It is possible there was a problem with one or more of the stocks.

Exploration of available GAL4 levels in the genetic system

Previous results from other thesis students suggested that using the GAL4/UAS driver to overexpress *CHD1* while also using it to direct knockdowns through RNAi could cause a titration of GAL4 away from UAS-CHD1, thus influencing the severity of the wing vein phenotype (Sharon Kim, ’13). In order to further explore the limits of the GAL4 system, I crossed the recombinant stock containing both the *UAS-CHD1* and *69B-GAL4* transgenes to a stock containing another P-element directing GAL4 production (also *69B-GAL4*). If GAL4 is limiting, I would expect a more severe wing defect phenotype upon the addition of more GAL4. This cross yielded flies with wing defects that were neither enhanced nor suppressed (Figure 9B). These crosses were performed at 24 ˚C, but were not performed again at 29 ˚C. This suggests that GAL4 is not limiting.

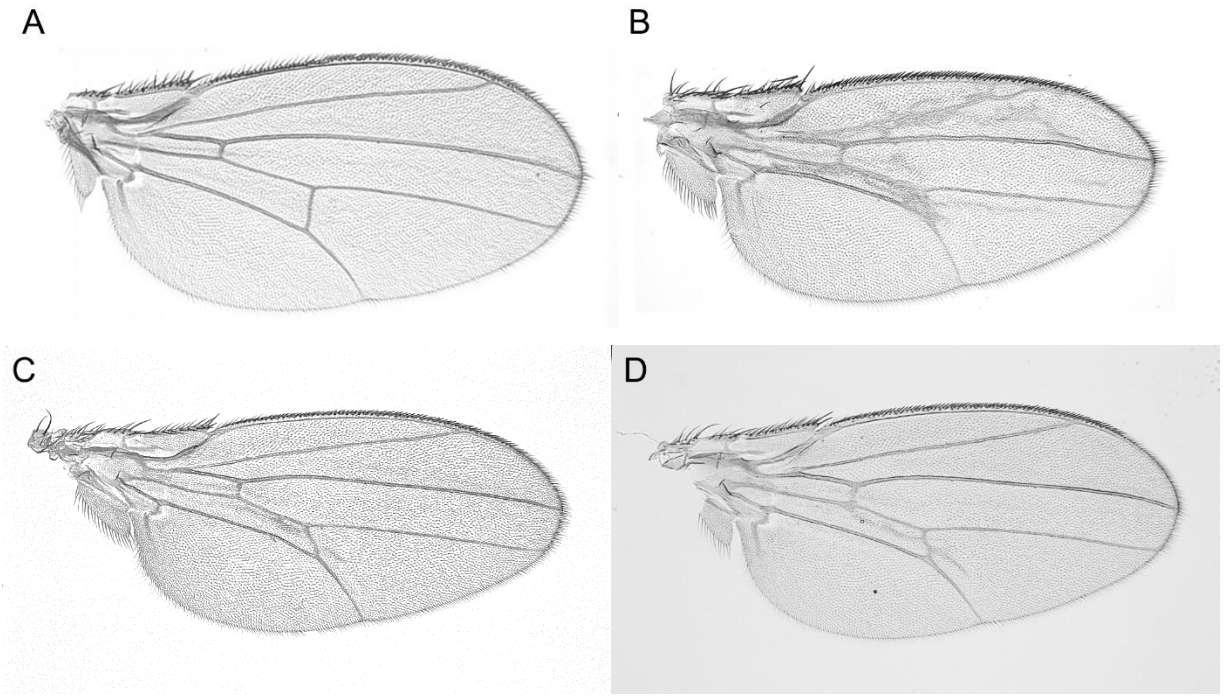

Figure 8. GAL4 is not limiting in the genetic system. (A) Wild-type wing (*OregonR).* (B) Wing vein defects caused by overexpression of *CHD1* alone (*w; P[w*$^{+}$*, UAS-chd1+]113/ P[w*$^{+}$*,69B-GAL4](R2*). (C) Wing vein defects caused by overexpression of *CHD1* and knockdown of *mCherry* at 24 ˚C (*w; P[w*$^{+}$*, UAS-chd1+]113, P[w*$^{+}$*,69B-GAL4](R2)/RNAi-mCherry*). (D) Wing vein defects are not changed following expression of additional *GAL4* at 24 ˚C (*w; P[w*$^{+}$*, UAS-chd1+]113, P[w*$^{+}$*,69B-GAL4](R2)/P{w[*$^{+}$*mW.hs]=GawB}69B-Gal4).*

Table 5. Summary of crosses with *CHD1* overexpression

	24 ·C		29 ·C	
	Result	**No. of Flies**	**Result**	**No. of Flies**
RNAi-mCherry	**Control (24 ˚C)**	**34**	**Control (29 ˚C)**	**40***
RNAi-INO80	**No Change**	**18**	**Enhanced**	**1****
69B-GAL4	**No Change**	**12**	**ND**	**ND**

ND indicates crosses not performed at given temperature
*39 female flies and 1 male fly
**This fly was female

Examining CHD1 and H1 functional interactions through wing-based genetic assay system

As mentioned, previous research indicates a potential interaction between CHD1 and H1, but the nature of the interaction remains unclear. To how CHD1 and H1 functionally interact, I performed a cross in which *CHD1* was overexpressed while *H1* was knocked down. I used two different *H1* lines to confirm that findings can be replicated. Crosses performed at 24 ˚C showed significantly enhanced wing vein defect phenotypes. Crosses performed at 29 ˚C did not produce any viable progeny with the desired genotypes (Figure 10B, C).

To test if *H1* knockdown by itself results in wing vein defects, I preformed crosses in which *H1* was knocked down without the overexpression of *CHD1*. Progeny from this cross had wild-type wings, indicating that RNAi knockdown of *H1* does not cause changes in the severity of wing defects by itself. These crosses were performed at 24 ˚ (Figure 10D, E) and were not repeated at 29 ˚C. This is supportive of a functional interaction between CHD1 and H1, suggesting that the proteins may function antagonistically towards each other.

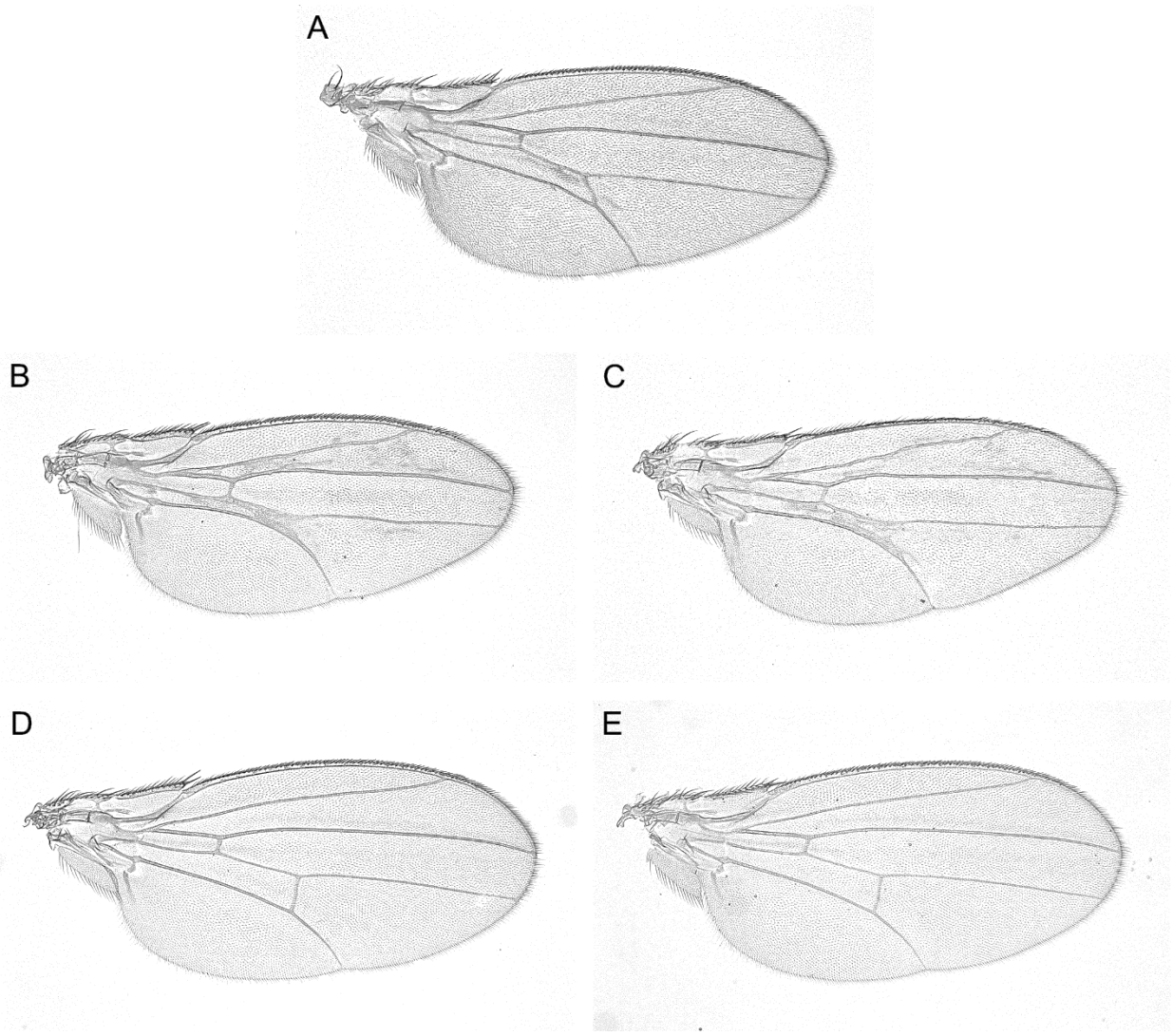

Figure 9. Knockdown of *H1* with simultaneous overexpression of *CHD1* enhances wing vein defects. (A) Overexpressing *CHD1* with *mCherry* knockdown contol *(w; P[w+, UAS-chd1+]113, P[w+,69B-GAL4](R2)/RNAi-mCherry)*. (B) *H1* knockdown with *CHD1* overexpression (*w; P[w+, UAS-chd1+]113, P[w+,69B-GAL4](R2)/UAS H1RNAi8-4).* (C) *H1* knockdown with *CHD1* overexpression (*w; P[w+, UAS-chd1+]113, P[w+,69B-GAL4](R2)/UAS H1RNAi10-3).* (D) *H1* knockdown by itself does not produce wing vein defects *(P[w+,69B-GAL4](R2)/UAS H1RNAi8-4).* (E) *H1* knockdown by itself does not produce wing vein defects (*P[w+,69B-GAL4](R2)/UAS H1RNAi10-3*).

Discussion

In this project, I investigated the interaction between transcription factors H1 and CHD1 using a genetic assay that takes advantage of a wing vein defect phenotype caused by *CHD1* overexpression. These tests aimed to expand on previous students' research, specifically examining a proposed working model for H1 and CHD1 interactions and exploring the limits of the GAL4/UAS system used in these experiments.

Understanding the behavior of the GAL4/UAS system

We are using the GAL4/UAS system to simultaneously overexpress *CHD1* and knock down target genes. I designed genetic experiments to better understand the behavior of the system (Sharon Kim, Scripps '13). First, I repeated two of Sharon's crosses to test if the GAL4/UAS system functioned similarly in my experiment as it did in hers. Crosses that knocked down *INO80* and *mCherry* at 24 ˚C produced wing vein defect phenotypes that were not consistent with Sharon's data. As I could not access the laboratory for over a year due to the Covid pandemic, I was unable to explore this further.

Existing research indicates that the GAL4/UAS system may have variable or diminished expression over time (Halpern et al., 2008). Surprisingly, progressive diminishment in GAL4-mediated gene expression has been observed in tobacco plants as the plants matured, potentially due to the methylation of the GAL4 binding site (Galweiler et al., 2000). In *Drosophila*¸ an investigation into the NAD-dependent modifying enzyme, poly(ADP-ribose) polymerase (PARP), and the NAD+-dependent histone deacetylase, SIR2, revealed that both PARP and SIR2 may alter the chromatin structure of sequences that are prone to be silenced during development (Tulin et al., 2002). Researchers involved in the analysis of the PARP and SIR2 proteins use epitope-tagged mitochondrial proteins to observe changes in the expression of *UAS* and *GAL4*

constructs, noting that the levels of PARP and SIR2 may be affecting the variegated expression of the *GAL4/UAS* constructs. Of particular relevance to this project is that the variegated expression of proteins mediated by the *69B-GAL4* driver is almost completely suppressed by a mutation that disrupts gene expression in only one copy of the *SIRS2* gene (Tulin et al., 2002). This suggests that there may be an external factor that is affecting the performance of the GAL4/UAS system that I had not previously accounted for.

In my project, the recombinant stock that contains the *UAS-CHD1* and *69B-GAL4* elements was balanced using *GAL80*. Since GAL4 activity is antagonized by GAL80, I had assumed that the constructed transgenic line is stable. However, the evidence regarding a potential diminishing of the GAL4/UAS system over time, or variation in the expression of the system presents complications that I did not previously consider. These complications with the activity of the system may have led to the reduction in the strength of the wing vein defect phenotypes in flies that overexpress *CHD1* over time. One solution is to out-cross the fly to wild-type stocks to remove second-side modifiers of the GAL4/UAS expression system. This could be done once the lab reopens.

However, it is also notable that both the crosses knocking down *mCherry* and *INO80* produced wing vein defects that were much more consistent with Sharon Kim's thesis when completed at 29 ˚C. The reduced viability of male flies in these crosses is similarly consistent with Sharon's findings regarding the increased penetrance of wing vein defect phenotypes in male flies (Sharon Kim, Scripps '13). Both the reduced viability in male flies and the increased penetrance of wing vein defects can be attributed to GAL4's higher activity at 29 ˚C (Brand and Perimmon, 1994). While I cannot currently explain the unexpected results arising from crosses

that included *RNAi-trx* and *RNAi-ISWI*, results from the crosses knocking down of *mCherry* and *INO80* suggest that, even if the GAL4/UAS system diminished over time, it is still functioning.

Next, I aimed to build further on Sharon Kim's work by testing her model for the titration of GAL4 away from *UAS-CHD1* by *UAS-RNAi*. I proposed that, if GAL4 is limiting, the introduction of more GAL4 would produce more severe wing vein defect phenotypes. However, severity of wing vein defects visible in flies with additional GAL4 was not notably different than the control cross at 24 ˚C. It is possible that the amount of GAL4 expressed by the *69B*-driver is sufficient to allow maximal *CHD1* expression from the *UAS* transgene. If that were the case, additional GAL4 would have no effect. If, at the same time, there is not excess GAL4, then a reduction in GAL4 activity at the *UAS-CHD1* element resulting from the introduction of additional UAS sites (driving the small hairpin RNAs) could result in a decrease in the wing phenotype caused by *CHD1* overexpression. Given GAL4's increased activity at 29 ˚C, it could be informative to repeat this cross at 29 ˚C, which could be done when students are allowed to return to the lab.

Establishment of a working model of CHD1 and H1 interaction

As previously stated, the remainder of my work focused on understanding the interaction between CHD1 and H1, building off of the results obtained a Vicky Lu, a previous student in the Armstrong lab. Vicky used *in vivo* analyses of *Drosophila* polytene chromosomes to visualize H1-GFP on the chromosome of flies expressing varying levels of CHD1. She observed that RNAi-mediated knockdown of *CHD1* had no effect on levels of H1-GFP, while *CHD1* overexpression resulted in reduced levels of H1-GFP (Vicky Lu, Scripps '20). These results indicate that excess CHD1 binding to linker DNA may be evicting H1.

Given Vicky's results, it is noteworthy that Ocampo et al. found that CHD1 and ISWI (another ATP-dependent chromatin remodeler) compete to direct nucleosome spacing in *S. cerevisiae*, with heavily transcribed genes demonstrating either extremely short or extremely long spacing, with H1 binding increasing with greater nucleosome spacing (Ocampo et al., 2016). These researchers further predict that CHD1 directing shorter nucleosome spacing results in the eviction of H1, allowing chromatin unfolding (Ocampo et al., 2016). Taken together with Vicky's results, this information is further informative of a model of CHD1 and H1 interactions in which CHD1 binding counters H1 deposition.

Biophysical studies of CHD1 and H1 produce further evidence consistent with the hypothesis that CHD1 and H1 physically compete with each other. Specifically, cryo-electron microscopy showed yeast CHD1 binding at superhelical location (SHL) +2 between extranucleosomal DNA and the second DNA gyre. Investigation reveals that CHD1 binds via its SANT and SLIDE DNA-binding domains at linker DNA (Farnung et al., 2013). In *Drosophila* H1, an NMR study of H1-nucleosome complexes suggested H1 uses two distinct regions in its C-terminal tail for nucleosome binding and chromatin structure condensation (Zhou et al., 2013), binding at a similar location as CHD1. The study proposes that H1 bridges the nucleosome core and linker DNA at two distinct domains (Zhou et al., 2013).

These studies indicate that CHD1 and H1 share binding sites at similar areas of the chromatosome, supporting the hypothesis that CHD1 and H1 interact competitively (Figure 12). Moreover, crystal structures in which CHD1 was bound to the nucleosome were altered compared to structures in which CHD1 was absent in that, in the presence of CHD1, two turns of nucleosomal DNA were detached at SHL -5 and -7 while extranucleosomal DNA was rotated ~60˚, incompatible with H1 binding (Farnung et al., 2013). These findings together with Vicky's

work led to the proposal of a working model in which CHD1 and H1 physically compete to bind to linker DNA. Specifically, Vicky proposed that CHD1 acts antagonistically to H1 to generate shorter nucleosome arrays and promote eukaryotic chromatin spacing (Vicky Lu, Senior Thesis, Scripps ’20).

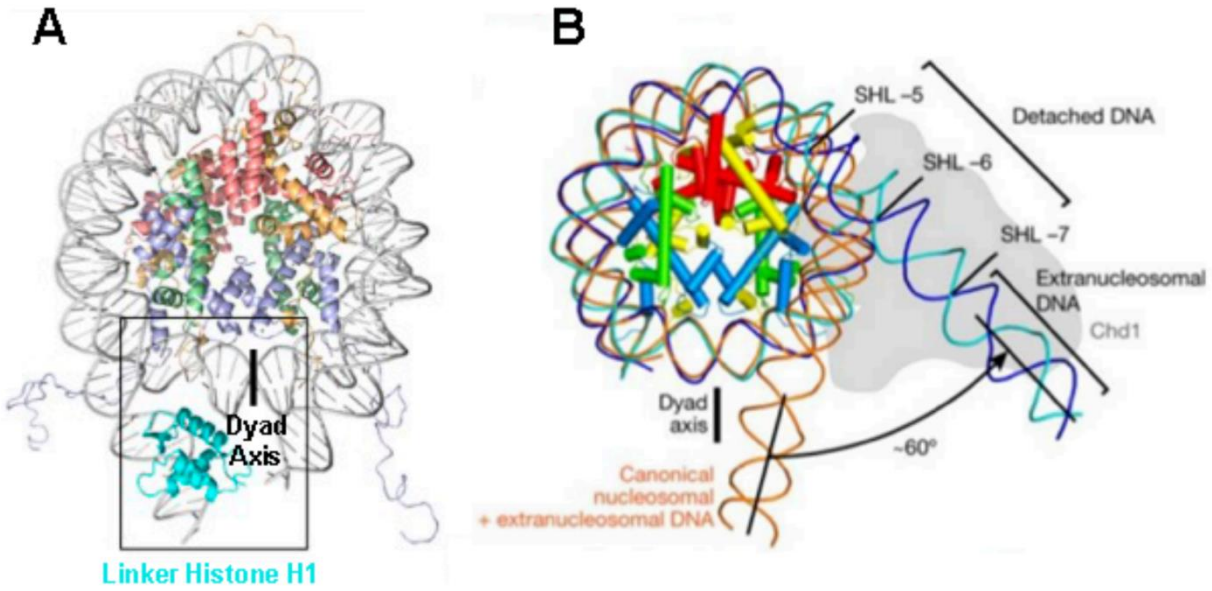

Figure 12. Structures of H1 and CHD1 binding to chromatosome in similar locations. (A) Structure of H1-chromatosome complex exhibiting asymmetric binding via two distinct domains. H1, H2A, H2B, H3, H4, and DNA are in teal, yellow, light red, purple, green, and gray, respectively. (B) Binding of CHD1 (gray) rotates extranucleosomal DNA ~60˚ (blue) with respect to its location in the absence of CHD1 (orange) (Figure from Vicky Lu, Senior Thesis, Scripps ’21).

Investigating the working model

My experiments build on the results of Vicky and Sharon’s work by using a novel, wing-based genetic assay to further study the interaction between CHD1 and H1. This assay uses the wing vein defect phenotypes produced by the overexpression of *CHD1* as a phenotypic marker for identifying CHD1’s functional partners. In my experiment, I used this assay to test Vicky’s working model. If the model is correct and CHD1 and H1 interact competitively, the wing vein

defect phenotype caused by *CHD1* overexpression would be enhanced with the simultaneous knockdown of *H1*. Alternatively, if CHD1 and H1 work together towards a similar function, *H1* knockdown would lead to a suppression of the wing vein defect phenotype. My genetic approach to investigating this interaction revealed that fly crosses in which CHD1 was overexpressed while H1 was knocked down produced significantly more severe wing defect phenotypes compared to the control. These results confirm Vicky Lu's proposal regarding CHD1 and H1 acting competitively and allow us to expand on the model.

Notably, a study performed using micrococcal nuclease digestion assays to study *D. melanogaster* CHD1 (Lusser et al., 2005). In the absence of H1, researchers observed CHD1-directed nucleosome repeat lengths of ~162 bp. Upon the induction of H1, the micrococcal nuclease digestion pattern became smeary, indicating a disruption of the periodicity of the nucleosomes that may be due to random association of the free H1 with nucleosomes, and suggesting that CHD1 is not able to assemble H1-containing chromatin *in vitro (*Lusser et al. 2005). In light of this information, further interpreting the cryo-electron microscopy data that illustrates the shared binding locations of CHD1 and H1 may be supportive of a model of CHD1 and H1 interactions in which H1 that is bound to the chromosome may be physically hindering CHD1 binding (Farnung et al., 2013; Zhou et al., 2013).

My results show that H1 knockdown with simultaneous CHD1 overexpression result in more severe wing defect phenotypes, indicating that CHD1 and H1 are acting in opposition. Specifically, my results suggest that CHD1 is able to bind more frequently to linker DNA in the absence of H1, suggesting that H1 being bound to the chromatosome is able to exclude CHD1. Taken in conjunction, these results allow us to expand on Vicky's working model that CHD1 is countering H1, by allowing the additional possibility of H1 preventing CHD1 from binding as

well. In this updated working model, I propose that H1 knockdown allows for increased CHD1 binding, with H1 binding to the chromatosome countering CHD1 deposition (Figure 12).

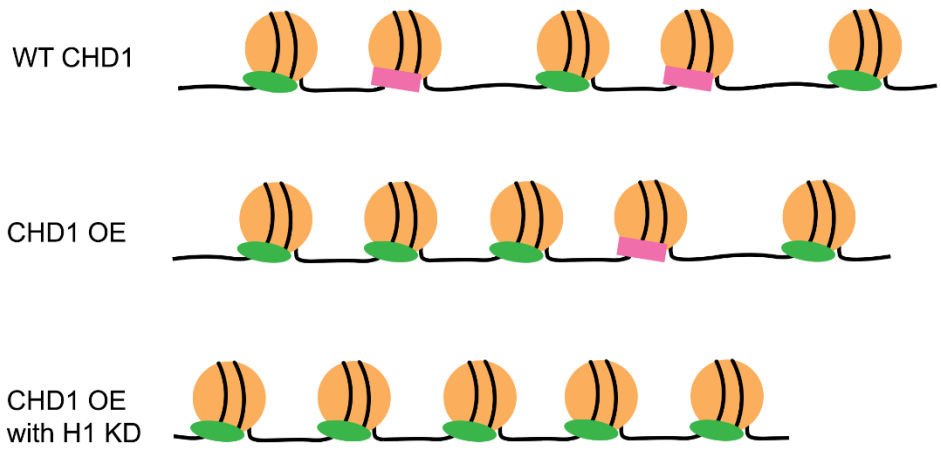

Figure 13. H1 knockdown allows increased CHD1 binding. Schematic model demonstrating CHD1 binding in flies with wild-type CHD1, CHD1 overexpression, and CHD1 overexpression with simultaneous H1 knockdown. Nucleosome core is represented with orange circles, CHD1 is represented with green ovals, and H1 is represented with pink rectangles. Shorter nucleosome spacing corresponding with CHD1 binding.

Further Questions and Implications

In future studies, I would clarify that RNAi is knocking down *H1*, in order to ensure that the system is working as we predict it to be. I would cross fly lines that contain the *H1-RNAi* transgene to fly lines containing H1 tagged with GFP to visualize the location of H1 on the chromosome. This would allow me to confirm if the results that we are seeing are due to the interaction between H1 and CHD1. We currently have the fly lines in the lab to do these experiments. Moreover, in order to account for potential off-target effects of using the RNAi system, it could be valuable to repeat this experiment with other lines in which *H1* is being knocked down. This would help ensure that changes in the severity of the wing vein phenotype that we are seeing are due to a functional interaction, rather than an artifact of the system.

However, my results were repeated with two distinct H1 lines, with overlapping targets for knockdown. Last, as mentioned above, to account for potential second-site modifiers in the recombinant stock containing the *GAL4/UAS* transgenes, I would out-cross the fly to wild-type stocks. These further crosses could be done once the lab reopens and would be valuable controls to ensure that the system is functioning properly.

It is also important to note that, when considering this model, we are assuming that it is the result of a direct interaction. Thus, it is necessary to point out that these results may be the result of an indirect interaction, such as a shared transcriptional regulatory program (Kavi et al., 2018). To further clarify the interactions between CHD1 and H1, it is necessary to understand the transient nature of CHD1 and H1 binding; how long they may be bound to the chromosome. As our working model predicts CHD1 and H1 to be physically competing to bind to extranucleosomal DNA, it is important to also understand if and how their different residence times affect their interactions. Future investigations into these topics could be helpful in clarifying our working model for CHD1-H1 interactions. FRAP studies could begin to get at these questions.

Alternatively, it is possible that the knockdown of *H1* could be affecting the expression of the GAL4/UAS system itself, thus affecting CHD1 levels (Tulin et al., 2002). Tulin et al. observed changes in the expression of the *GAL4/UAS* construct as a result of altered PRAP and SIR2 protein levels (Tulin et al., 2002). As our current model predicts that CHD1 and H1 are competing on the level of protein-protein interactions, it would be valuable to clarify if *H1* knockdown by itself was causing any changes in chromosome structure, possibly affecting the transcription of *CHD1*. This could be investigated by using western blot and RT-qPCR to determine how much CHD1 is being expressed in flies that simultaneously knock down H1.

CHD1 is an incredibly important element of chromosome structure and plays a crucial role in regulating gene expression. Understanding more about its function, and particularly its functional partners can be influential in helping us learn more about the diseases and conditions associated with CHD1. My results suggest that CHD1 and H1 interact competitively, with H1 binding preventing CHD1 deposition, identified through a wing-based genetic assay using *D. melanogaster*. These findings can be more broadly applied to humans and organisms in which CHD1 similarly directs nucleosome structure to help us learn more about our health.

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