This summer, I had the fantastic opporn opporn oppor20 -83.D59.5 (oppor) 239.5 9.2 (,) 1083.3 (f) -0.3 (a) -21.734 ( ) -83.D59.5 to determine

s activity determines its nuclear location or if a gene’s nuclear location dictates its genetic activity.

HP1 and Condensin II are structural proteins that have been associated with changes in chromatin conformation. HP1, or Heterochromatin Protein 1, is a homo-dimerizing protein that is capable of inactivating regions of a chromosome and is responsible for the transition from euchromatin to heterochromatin (reviewed in Zeng 2010). HP1 is a major component of gene silencing, which is an important part of the looping mechanism described above. Condensin II, on the other hand, is responsible for the architectural changes that allow chromosomes to separate from one another and to associate with themselves (Bauer 2012). This anti-pairing of homologous chromosomes is also an important component of the looping mechanism described previously. Both proteins play a role in the creation of chromosome territories.

To investigate the functions of HP1 and Condensin II in this system, we chose to use S2R+ fruit fly cell lines because they are easy to maintain and manipulate. We were able to obtain a cell line with an insertion of a repetitive sequence that allowed us to target silencing to a specific and known portion of chromosome 3, allowing us to control the sequence of events that leads to looping and associat (a) -1 (n) itarg

3. Then, we used DNA-Fluorescent In-Situ Hybridization (DNA-FISH) on those cells to determine if our targeted silenced region was associating with heterochromatin on its own chromosome, insinuating that looping is dependent on HP1 (Figure 2). Our baseline measurements were cells that did not express the HP1 protein.

Our analysis—for both experiments one and two—included measurements of the standardized distance between two heterochromatin compartments, one on the chromosome arm and one on the centromere; the proportion of cells in a given population that showed colocalization of e(e) -8.4 (t) 16.1 (e -131 (p)(hr) -0.3 h) 23.8 (r) -0.3 (om) 16.1 (n) 23.8 (42 100 -852 Tm /TT21 Tf compartment to the (pa) -8.4 nuclear periphery.

We ran experiment one under heat-stress conditions, inadvertently, for the first part of the summer. For results, we saw a significant difference in the standardized distances between the heterochromatin compartments, with HP1-expressing cells having smaller standardized distances than our baseline cells ($p<0.0001$). However, the other two measurements did not show statistically significant differences. We were able to rerun the experiment under regular conditions in the later part of the summer, but at this point, the purity of the antibiotic we were using to select for healthy cells was compromised and we experienced contamination in our cell. This introduced unexpected and unknown effects that we were unable to control for.

In experiment two, we wanted to determine if Condensin II contributes to the looping mechanism by antagonizing interchromosomal interactions and supporting intrachromosomal interactions. Our approach was to knockdown CapH2, the rate-limiting component of Condensin II, through RNA interference (RNAi) and to use HP1-expressing cells to determine if association of heterochromatin compartments is reduced when Condensin II is knocked down. Our baseline measurements were cells that expressed Condensin II (Figure 3).

For experiment two, we needed to generate double stranded RNA of the CapH2 sequence in order to knock Condensin II down. We were only able to run this experiment once due to time-restrictions and the cell populations had been growing under stress from bacteria-contaminated media. In order to remedy the stressful conditions, we ran a purification sequence prior to the knockdown which may have affected our results. In our only run, we saw no significant differences in any of our measurements. Verification of these results will require a careful re-run of this experiment.

In the lab’s first year undertaking this investigation, I was able to run through all of the protocols required for experiments one and two and fine-tune the procedures .4 ( ) f [(e) (s)8(l) -7.7 3up -8.4 (nd) 24 (o) 23.8 (l) -24 0 0 0.24 12 589.92 cm BT 42 0 0 42 238 -2007 Tm /TT4 (r

In the future, I would like to rerun experiments one and two with healthy cells, pure antibiotic, and fresh media. Additionally, I would like to create a DNA-FISH+antibody-labeling protocol that would allow me to obtain measurements specifically for cells that are expressing HP1 in the HP1-expressing cell populations. Lastly, I am excited at the prospect of working further with this system, possible shifting my focus to the effects of overexpressing Condensin II instead of knocking it down in order to understand its functions more fully.

Figure 1. Schematic of experiment one, showing how we will control the sequence of events in order to determine the functional roles of HP1 and Condensin II. 1) We target a region for silencing by localizing HP1 to a specified and known region. 2) We silence the region of interest and observe one of two results— 3a) The silenced region of interest will associate with heterochromatin on its own chromosome by looping or 3b) the silenced region of interest will be silenced but will not associate with heterochromatin on its own chromosome. HP1's role in looping will be determined.

Figure 2. DNA-FISH in experiment one. Probes for the dodeca satellite sequence on the centromeric heterochromatin (red flash) and for the LacO array targeted for HP1 induced silencing (orange flash) will be used to determine the location of two heterochromatin regions of interest. The probes will be visualized with a confocal microscope and distance analysis will be completed with TANGO, a distance analysis plug-in on the Fiji app. Colocalization will be determined by these measurements.

Figure 3. Schematic model of possible results for RNAi knockdown experiment. Wt represents wild-type results for a baseline HP1-expressing population of cells, where targeted silencing has brought the two heterochromatin regions of interest closer by looping. CapH2 RNAi knockdown