1) You have created P element transformants of a construct that contains the mini-white gene, which confers an orange eye color in a homozygous white mutant background. For each line you performed the following crosses:

- Select an initial orange-eyed female transformant and cross this male to w; CyO/Sp; TM3/Dp males.
- Select one orange eyed male progeny that carries CyO and TM3; cross this male to females homozygous for white.
- Score the resulting progeny for the presence or absence of the mini-white gene and the two balancer chromosomes.

Below are the results you get for each line. What chromosome is each P element in?

a) Stock 4: 126 flies total: 120 are P[w+]: 64 are CyO; 56 are TM3; all are females.
   132 are w-: 68 are CyO; 64 are TM3; all are males.

b) Stock 3 - 110 flies total: 114 are P[w+]: 56 carry TM3; none are CyO
   106 are w-: 32 are TM3; all are CyO.

c) Stock 6: 216 total flies 150 are P[w+]: 76 are TM3; 24 are CyO; 100 are female.
   66 are w-: all are CyO; 36 are TM3; all are male.

d) Stock 7: 100 flies total 74 flies are P[w+]: 26 are neither CyO nor TM3; 24 are TM3 and 24 are CyO.
   26 are w-: All are CyO and TM3
2) Genomic disorders arise due to structural features common to the human genome, which predispose for specific and recurrent chromosomal rearrangements.

a) Given the presence of the specific repeat sequences indicated below, (i) what types of chromosomal rearrangements would arise from intra-chromosomal non-allelic but homologous recombination events between the red repeats and between the yellow repeats, and (ii) what types of chromosomal rearrangements would arise from an inter-chromosomal recombination event between the first and second red repeat?

3) The achaete and scute genes encode two basic helix-loop-helix transcription factors. These genes are expressed in identical expression patterns and reside next to each other in the X chromosome about 25 Kb from each other. Both genes promote neural precursor development. An inversion breakpoint, In(1)sc[8], maps to the intergenic DNA about midway between achaete and scute. Embryos homozygous or hemizygous for this inversion express both achaete and scute in completely complementary patterns: that is, achaete and scute are now each expressed in exactly half of their wild-type expression pattern and they exhibit zero co-expression. In the regions in which these genes are expressed, they are now expressed at higher levels than in wild-type.

Provide a molecular explanation of this result.

Also, provide an explanation for why In(1)sc[8] flies exhibit wild-type neural precursor development in the face of overt changes to the expression of *achaete* and *scute*.
4) The hippo/warts signaling pathway is a relatively newly discovered signaling pathway that regulates cell proliferation and cell death in flies. Mutations in hippo, salvador, or warts lead to increased cell proliferation, decreased cell death, and cause at the molecular level a cell-autonomous upregulation of the levels of the Expanded protein (red in figure). The molecular signal that triggers activation of this pathway remains elusive, as hippo, salvador and warts all function in the signal-receiving cell to mediate signal transduction.

In a genetic screen you identify mutations in two new genes (genes A and B) that yield mutant phenotypes identical to those of hippo, salvador and warts. These mutations complement each other as well as mutations in all known members of the hippo/warts signaling pathway. To determine if either gene functions in the signal-sending cell, you use FLP/FRT-based methods to create mitotic clones homozygous mutant for each gene. You use GFP as your cell-autonomous marker to identify mutant clones unambiguously by their lack of GFP expression (a transgene is used to drive GFP under the control of a ubiquitously expressed promoter), and you assess Expanded protein levels within the mutant clones. Being a careful scientist, you also create mitotic clones of a wild-type chromosome as your control. The results of the clonal analysis are shown in the accompanying figure. Please answer the following questions.

A) Which gene functions in a cell autonomous manner and why?

B) Which gene functions in a cell non-autonomous manner and why?

C) Which gene is the better candidate to encode the sought after signaling molecule that activates the hippo pathway.
Expanded protein: red
darker red = more protein

GFP: green; Mutant clone: no GFP

Wild type control | Gene A | Gene B

Gene A and Gene B

1

2

Merge of GFP and Expanded
D) 1. What is the genotype of the cells (dark green) marked by arrow #1.
2. What is the genotype of the cells (light green) marked by arrow #2.

(Assume gene B is in the left arm of chromosome 2; please include all possible relevant information: e.g. FRTs, GFP, centromere…).

5) We are conducting a forward genetic screen to identify genes that regulate post-embryonic neurogenesis. We are mutagenizing an isogenic, lethal-free second chromosome that carries recessive visible mutations in cinnabar, brown, and speck. We are screening for mutations that when made homozygous alter the expression pattern of the transcription factor Hb9 in the thoracic region of the nerve cord; normally, Hb9 is expressed in 18 clusters of neurons in this region. To date, we have identified over thirty mutations (or more accurately mutagenized second chromosomes) that when homozygous yield obvious defects to the expression pattern of Hb9 in the CNS. Of note, larvae heterozygous for each of the mutagenized chromosome and the unmutagenized isogenic, lethal-free chromosome display a wild-type CNS phenotype.

One mutagenized chromosome yields an interesting phenotype: a variable number of Hb9 clusters are absent from most larvae homozygous mutant for this chromosome. A representative set of ten homozygous mutant larvae yield the following phenotype with respect to the presence/absence of Hb9 clusters:

1) 6/18 Hb9-positive clusters are missing
2) 1/18 Hb9-positive clusters are missing
3) 12/18 Hb9-positive clusters are missing
4) 18/18 Hb9-positive clusters are missing
5) 318 Hb9-positive clusters are missing
6) 5/18 Hb9-positive clusters are missing
7) 16/18 Hb9-positive clusters are missing
8) 0/18 Hb9-positive clusters are missing
9) 918 Hb9-positive clusters are missing
10) 13/18 Hb9-positive clusters are missing

a) What is the penetrance of this phenotype?

b) What is the expressivity of this phenotype?
We performed complementation crosses between this mutant line, which we call K146, and the second chromosomal deficiency kit, which contains ~190 individual fly lines that collectively uncover over 95% of the second chromosome. FYI: the K146 chromosome is carried over CyO; all deficiency lines are also carried over CyO. Our results are as follows:

- Df(2L)ED1272: 0/80 adult flies display straight wings.
- Df(2L) ED1203: 13/32 adult flies display straight wings.
- Df(2L)ED1303: 6/17 adult flies display straight wings.

(Here, it will help to go to FLYBASE (http://flybase.org/). You can find the deficiencies by going to “Links” -> “Stock Collections” -> “Bloomington” and searching for the deficiency by its name. From the stock, you can get the cytology of the deficiency and the genes deleted. At this point, I’d click on one of the genes to open the flybase page for it, on the left click on Gbrowse or JBrowse to get a genome viewer (I think I prefer Jbrowse to view the genome and then tweak settings so you can see a relatively large region of the genome, and toggle deficiencies so that you see them).

c) How do you interpret these results?

All larvae of the genotype K146/Df(2L)ED1272 lack all Hb9 clusters and exhibit a nerve cord itself much reduced in size relative to K146 homozygous larvae.

d) From these data, what would you interpret about the nature of the affected allele?
  - In your answer, refer both to Muller’s classification scheme for mutant alleles (Tim went over this, and I always ask questions in prelims on this) and to the likely molecular nature of the allele.
6) In a similar EMS-based saturation mutagenesis, we identified a single third chromosomal lethal mutation that when homozygous resulted in a dramatic perturbation to CNS structure. As a first step towards identifying the affected gene, we mapped the lethal mutation relative to four P[w+] P elements of defined location using the following cross scheme:

Go  \( P[w+]/P[w+] \)  \( \times \) lethal(3)/TM6 Tb

F1  \( P[w+]/\) lethal(3) virgin females  \( \times \) lethal(3)/TM6 Tb males

F2: recombinant chromosome/lethal(3)

In the F2 generation, we scored the eye color of adult flies of this genotype: recombinant chromosome/lethal(3)

The results we obtained for each of the four P elements are shown below:

**P[w+]BG00690 Genetic map position 40.5**

Results: 44 white-eyed progeny and 186 P[w+] progeny

**P[w+]Alh[BG02270] Genetic map position 47.5**

Results: 13 white-eyed progeny and 115 P[w+] progeny

**P[w+]cher[BG02734] Genetic map position 60.0**

Results: 6 white-eyed progeny and 279 P[w+] progeny

**P[w+]BG01881 Genetic map position 72.4**

Results: 38 white-eyed progeny and 184 P[w+] progeny

a) Based on the above data estimate the genetic map position of the lethal mutation?

Hint: If you are struggling, draw out the chromosomes for the crosses.
b) Once we localized the mutation to an approximate genomic location, we used deficiency mapping to localize it further. In each complementation test, we carried out the following cross –

\[ Df(3R)XYZ/TM6 Tb \times lethal(3)/TM6 Tb \] – and scored for the presence of \( Df(3R)XYZ/TM6 Tb \) adult progeny (flies that lack \( TM6 Tb \)). Based on the below detailed results, draw a genomic map of the region and delimit the smallest genomic region within which the lethal mutation resides. How many genes are in the identified region? (Here, it will help to go to FLYBASE (http://flybase.org/). You can find the deficiencies by going to “Links” -> “Stock Collections” -> “Bloomington” and searching for the deficiency. From the stock, you can get the cytology of the deficiency and the genes deleted. At this point, I’d click on one of the genes to open the flybase page for it, on the left click on Gbrowse or JBrowse to get a genome viewer (I think I prefer Jbrowse to view the genome and then tweak settings so you can see a relatively large region of the genome, and toggle deficiencies so that you see them).

\[ Df(3R)BSC750: \quad 32/111 \text{ progeny were non-TM6 Tb adults} \]
\[ Df(3R)BSC741: \quad 22/53 \text{ progeny were non-TM6 Tb adults} \]
\[ Df(3R)ED5664: \quad 19/82 \text{ progeny were non-TM6 Tb adults} \]
\[ Df(3R)Exel6174: \quad 0/104 \text{ progeny were non-TM6 Tb adults} \]
\[ Df(3R)BSC515: \quad 39/99 \text{ progeny were non-TM6 Tb adults} \]
\[ Df(3R)BSC569: \quad 21/61 \text{ progeny were non-TM6 Tb adults} \]

c) Through complementation crosses with lethal alleles in most genes of this region, we identified two alleles in a single complementation group that failed to complement our lethal mutation. But, when we sequenced the entire open-reading-frame of the gene, we failed to identify any lesions. Assuming the gene is the correct one, provide two reasons that can explain the data.
d) We then sequenced the entire genomic region and obtained the sequence data on the following page for the 5’ UTR of the candidate gene.

Black non-highlighted DNA is chromosomal sequence that maps back to the reference genome. Green highlighted DNA is DNA sequence that does not map back to the reference genome using typical bioinformatics pipelines.

A9:
Left Junction

chromosomal sequence |------
ATTCGAATTCAAATAACTGGAATATAGAAAATATTCAAATTAAATCAGAATTTAACACTGTAATAGCT
ACTGGATGGATGTTTCTGATAGCGGAGGAGCTACTACCGAGCGAGTTAAGTCCGTGAAGGCTTTG
TGATAGATTTTTGTAGAGGCGTGTTGTTAGGCGGAGGTTGTTGTTG

Right Junction

-------- | chromosomal sequence
CGATTTTGTAGAGCCGGTTGTTGTTAGGCGGAGGTTGTTGTTG
CGTCCCCGGATTAGTTTCTGATAGCGGAGGAGCTACTACCGAGCGAGTTAAGTCCGTGAAGGCTTTG

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d1) What is the affected gene?
   - it is probably easiest to use BLAST via the FLYBASE website

d2) What do you think caused the lesion?
   - as above, but you will likely need to play around with the blastn options.

d3) What types of molecular lesions are most common with EMS-based mutagenesis?

d4) Why do you think an EMS-based mutagenesis led to such a lesion?