BIOL 463: Lonfat et al. Worksheet
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1. **It is quite common for research papers to have Figure 1 be the "most important" figure in the article. Consider Figure 1:**
    a. **What transgenic lines did the author use? Please briefly describe them (do these lines look somewhat familiar?)**
    The author used a lacZ reporter gene under *Hoxd9* regulatory control, that also contained a loxP element (which was complementary to an upstream loxP element in the *Itga 6* locus), inserted 1 Mb from the centromere. One line had expression of the Cre endonuclease to invert the floxed locus, the other did not. These lines are somewhat familiar insofar that they include *Hoxd9,* a gene we've studied in class, and the *Evx2* locus immediately upstream the HoxD cluster. They also make use of lacZ reporter technology we've seen before.

Good!

    b. **What do the data in Panel C show?**
    The data shows different lacZ reporter expression between maternally and paternally inherited *Hoxd9* alleles, in both inverted and non-inverted cell lines.

Can you be a bit more specific (details?) E.g. you see both intensity and distribution/location of the signal…

    c. **What is striking/unexpected about the data in Panel C?**
    The expression pattern for the non-inverted *Hoxd9* reporter did not change much between maternal and paternal *Hoxd9* alleles, whereas when the transgene was inverted, there was a profound change in expression pattern.

    d. **What direct conclusions do you make from the data?**
    Cre-mediated inversion of the transgenic locus is sufficient to abolish *Hoxd9* lacZ reporter expression in embryos with the maternal *Hoxd9* allele.

2. **How did the authors show that the peculiar effect observed is specific and position/site-specific? Do you agree with their data interpretation and with their conclusion?**
    The authors used another reporter construct (different promoter) to determine specificity of DNA sequence (*lacZ* sequence is necessary for imprinting effects). To determine position specificity, the authors inserted the reporter into various regions in a 2 Mb area around the *Itga6* locus, and determined no imprinting occurring. Their conclusions seem warranted from the data they generated; insofar that changing the *lacZ* reporter did not change expression between maternal and paternal alleles, its safe to conclude that the promoter is not sufficient to drive inhibit or induce imprinting. The same logic applied to their conclusions regarding the significance of the DNA sequence itself and the *Itga6* locus - because insertion into other nearby regions beyond the *Itga6* locus did not induce imprinting its reasonable to conclude that *Itga6* is necessary for allelic

And maybe something related to the hox promoter

imprinting in that chromosomal region; moreover, because different DNA sequences did not induce imprinting, it is safe to conclude that there is something necessary about the *lacZ* gene for imprinting.

3. **Consider Figure 2 (please don't hesitate to ask for clarification if you and your group have questions about it!):**
   a. **Briefly explain how to "read" the diagrams shown (i.e. what do the rows of circles represent, what do the white vs. black circles represent).**

what are the rows?

White circles represent cytosines that are unmethylated, and black circles show methylated cytosines. These patterns were determined using bisulphite sequencing, which provides data on cytosine methylation status.

   b. **What do the data in Figure 2 show?**

2A shows that there is differential methylation patterning in maternal/+ vs +/paternal with the Inv*(rel5-ltga6)* genotype.

2B shows that Inv*(rel5-ltga6)* escaper embryos do not follow the same methylation patterning - their methylation rate is strongly reduced and variable expression of the transgene results in varying methylation profile.

which ones have more/less?

2C shows that there is differential methylation patterning in sperm and oocytes with the Inv(*rel5-ltga6*) genotype.

2D shows that there is very similar methylation patterning in +/paternal and maternal/+ with the *rel5* genotype.

   c. **Why aren't there a "paternal/+" and a "maternal/+" groups for sperm and oocytes?**

Sperm only have a paternal lineage, and oocytes only a female, as they are male-specific and female-specific cell lines respectively. They do not contain a pair of chromosomes, only the Y (paternal) or X chromosome respectively.

plus all the autosomes

   d. **What are "escaper" embryos, and how were they identified prior to bisulphite sequencing?**

Escaper embryos are those that did not experience complete silencing on the maternal chromosome, to varying extents, from little lacZ staining/mostly methylated to almost no repression/methylation, like the parental transgenic organisms. Before bisulphite sequencing they were identified through the β-gal staining technique.

good summary and explanation

   e. **What can we directly conclude from the data?**

We can directly conclude that methylation is correlated with/plays a role in silencing of the maternal lineage, as the methylation profile corresponds with

which lineage is silenced, and varies in the escaper embryos and non-inverted animals. Good! Most people did not include this last piece of evidence

4. **Figure 3 depicts the results of a series of 4C experiments. Try to "read" the figure and see if you can identify the information described in the text.**
    a. **What did the authors do, and what are the results?**

Did the maternal transgene interact with the paternal? Or do you mean something else?

The authors performed a 4C chromosome conformation capture experiment to determine the interactions between the embedded *lacZ* locus neighboring chromatin regions. The chromatin interactions between maternal and paternal alleles were very similar, in that the most chromatin interactions, regardless of whether the allele was paternal or maternal, occurred around the *lacZ* locus were in the centromeric desert between the *Atf2* gene and the *HoxD* cluster. When the *lacZ/Itga6* locus is inverted, only the paternal allele interacts with digits enhancers.

We can't really say that the silencing part is sufficient (we don't know if the silencing or the distal chromatin interactions happen/don't happen first). However, the inversion that you describe is suffcient:)

    b. **What can be directly concluded from the data?**

Silencing of the *Itga6*-embedded *lacZ* gene via maternal imprinting after Cre-mediated *lacZ* inversion is sufficient to diminish distal chromatin interactions between digit enhancer loci (I-IV) and the *Itga6* locus.

5. **How does each figure support the statement in the title of the article? Which one supports it the most?** *(Statement from the article title: "Transgene and locus-dependent imprinting reveals allele-specific chromosome conformations")*
    a. **Figure 1** uses tissue staining to illustrate the differential expression of the *lacZ* reporter gene in +/Paternal vs Maternal/+ with both the *rel5* and (more clearly different) Inv*(rel5-Itga6)* genotypes. This shows the transcriptional effect of different imprinting patterns at the locus altered in this experiment.

Careful about equating high DNA methylation to tight packing - it needs more than just methylaiton to get packed tightly

    b. **Figure 2** shows how transgene imprinting corresponds with differing allelic DNA methylation profiles, supporting the idea that there are allele-specific chromosome conformations. The differential methylation profiles display how one chromosome is more tightly packed (ie. more methylation), whereas the other is unmethylated and remains free for transcription, two different conformations.

    c. **Figure 3** shows how maternal and paternal alleles differ in their interactions with distal digit enhancers after cre-mediated inversion of the *Itga6* locus. It demonstrates a direct difference in chromosome conformations between two states (active or silenced) of an imprinted locus.

Good!

    d. **Figure 4** demonstrates the difference in neighboring gene expression and interaction; with maternal silencing of the *Itga6* locus, digit enhancers are able to better associate with neighbouring *Dlx* genes. This demonstrates a clear difference in chromosome conformation between maternal and paternal alleles,

in that the silenced maternal allele compacts the chromatin at the transgenic locus, allowing the *Dlx* genes better access to the digit enhancers; with the paternal locus, there is more interaction between *Dlx* loci and the transgene.

So, which figure best supports, on its own, the title? (I know that it's a matter of opinion…)