In-class small assignment: Lonfat et al. paper

Assigned paper: Lonfat, N., Montavon, T., Jebb, D., Tschopp, P., Nguyen Huynh, T.H., Zakany, J., Duboule, D. (2013). Transgene- and locus-dependent imprinting reveals allele-specific chromosome conformations.

Names of your group members: Jasia Ho, Miguel Oreta, Oline Pade Jensen

Question 1

It is quite common for research papers to have Figure 1 be the "most important" figure in the article. Consider Figure 1:

- 1. What transgenic lines did the author use? Please briefly describe them(do these lines look somewhat familiar)?
- hoxd9lacZ reporter transgene in mouse model
- LacZ inserted several sites upstream of HoxD
- Furthermore they performed a 2.7 Mb inversion, which brought the transgene into the Itga6 gene
- They do look familiar because we have been talking about mouse models and hox genes and LacZ reporter genes
- 2. What do the data in Panel C show?
- Shows LacZ reporter expression in mice embryo with normal transgene and embryo with inverted transgene between maternal and paternal alleles
- Mice embryos with uninverted LacZ did not show a discernable difference between maternal and paternal inherited genes
- However, when the transgene is inverted, paternal mice show high expression levels of LacZ characterized by blue spread throughout their body whereas maternal mice show little to no expressions of LacZ, characterized by minimal blue in their body
- 3. What is striking/unexpected about the data in Panel C?
- In the inverted transgene mice, there is a drastic difference in lacZ expression in mice who inherited maternal vs paternal transgene. Paternally inherited transgenes had a much stronger LacZ expression, whereas, maternally inherited transgene had little to no expression of LacZ.
- It is surprising that there is a difference between maternal/paternal inherited transgenes for the inversion only
- 4. What direct conclusions do you make from the data?
- The inversion of the transgene is sufficient to induce differential expression of the LacZ reporter in maternal vs paternal inheritance.

Question 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?

- They looked at DNA methylation and histone methylation at specific loci in maternal vs paternal inheritance
- And used rt-qPCR with specific primers to determine expression levels
- Before the inversion the transgene showed no expression bias, but after the inversion it did show bias
- Yes, we do agree

Question 3

Consider Figure 2 (please don't hesitate to ask for clarification if you and your group have questions about it!):

- 1. 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).
- Circles = sites that could possibly be methylated
- Black = methylated already; white = unmethylated
- 2. What do the data in Figure 2 show?
- Oocytes and maternal+ have high methylation rates at the inv(rel5-Itga6), whereas the sperm and paternal+ have low methylation rates.
- Both Maternal+ and paternal+ have low methylation rates at rel5
- 3. Why aren't there a "paternal/+" and a "maternal/+" groups for sperm and oocytes?
- The sperm would only have had paternal influence (since it comes from the father)
- The oocyte would only have had maternal influence (since it comes from the mother)
- 4. What are "escaper" embryos, and how were they identified prior to bisulphite sequencing?
- Embryos whose maternal chromosomes were not completely silenced
- Had reduced expression patterns, with patches showing lacZ activity
- 5. What can we directly conclude from the data?
- Inversion of the transgene is sufficient to induce differential methylation patterns between maternal+ embryos/ oocytes and paternal+ embryos/sperm

Question 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.

- 1. What did the authors do, and what are the results?
- The authors conducted 4C analysis on the region around the lacZ transgene
- They could see that maternal and paternal alleles showed similar interactions with HoxD with the right-side-up transgene
- When the transgene was inverted, the paternal allele interacted much more with the HoxD enhancers than the maternal alleles did
- 2. What can be directly concluded from the data?

Inversion of the transgene is sufficient to induce differential interactions with enhancers between maternal and paternal alleles (paternal alleles interact more with the enhancers).

Question 5

How does each figure support the statement in the title of the article? Which one supports it most?

Title: Transgene- and locus-dependent imprinting reveals allele-specific chromosome conformations

Figure 1

- Figure 1 shows that maternally-derived alleles can be expressed differently from paternally-derived alleles of the same kind. The presence of the LacZ reporter allows visualization of the differences between the two alleles.

Figure 2

- Figure 2 shows that this difference in expression is likely caused by a difference in methylation patterns between the maternal/paternally-derived alleles. It confirms the specificity of the imprinting.

Figure 3

- Figure 3 shows that the difference in methylation causes differential association of the transgene with the enhancers. It reveals the differences in chromosome conformations specific to the different alleles.

We believe that Figure 3 is the most vital figure to their claim as it allows us to see that there is a difference in chromsome conformation when the samples have differently imprinted alleles.