Journal Club 3 Reading Guide

From the review article by Froberg, Yang and Lee, and from the book (pgs 443-444 and 601-606) and our lectures:

What are non-coding RNAs? How are these different from mRNAs?

Why is it necessary for one X chromosome to be inactivated in humans?

What is the name of the long non-coding RNA that is responsible for inactivation of the X chromosome?

What is the name of the long non-conding RNA that is responsible for marking the active X chromosome?

The long non-coding RNAs involved in X chromosome dosage regulation are said to act "*in cis*" - what does this mean?

Examine Figure 1 of the Froberg et al article, which gives an overview of X chromosome inactivation. Summarize this process in your own words.

Tsix is said to be the "antisense transcript" of Xist - what does this mean?

How might a "sense" transcript and an "antisense" transcript interact?

Examine Figure 2 of the article by Froberg et al: Where is the promoter for Xist? Where is the promoter for Tsix?

What is a Barr body?

About the article by Jiang et al:

1. What does "trisomy" mean? Look up and name a few human conditions caused by trisomy diseases. Which trisomies are the most common?

2. Jiang et al note in their introduction that it has been extremely difficult to find treatment for syndromes caused by chromosomal abnormalities (like Down's Syndrome) as opposed to those caused by a single gene (Like Cystic Fibrosis, Fragile X Syndrome, PKU, and others we have discussed in class). What are the additional challenges faced when trying to correct a chromosomal abnormality?

3. Jiang et al are attempting to develop "chromosome therapy" for trisomic diseases - what does this have to do with X chromosome inactivation? Explain what they are trying to accomplish in your own words.

4. The authors' first challenge was to integrate the Xist gene into Chr 21 in a precise location (targeted integration). What location where they shooting for, and why was this location chosen? Why is this first step itself an important accomplishment? (HINT: look at the first sentence under the heading "Insertion of XIST into a trisomic chromosome 21")

5. Figure 1a depicts the plan for the author's strategy. They are using iPS cells from a male patient with Trisomy 21. What are iPS cells?

6. The authors used Zinc-Finger Nucleases (ZFNs) to integrate Xist in a specific location. What are ZFNs? How are they similar to the technology we have previously discussed for targeting specific areas of the genome (CRISPR)?

7. How successful were they are targeting this specific location? (What percentage of the cloned cells expressed Xist in the same location as DYRK1A)?

8. What technique did the authors use to verify that the Xist gene was inserted into the DYRK1A gene on Chr 21? (Examine Figure 1c,d,e).

9. The Xist gene integrated into Chr21 is inducible by doxycycline. Does this mean that the addition of doxycycline turns the Xist gene on or off? How do inducible genes work? What region of the gene to they target?

10. Figure 2 shows that the copy of Chr21 containing the integrated Xist forms a Barr body. What measures do they use to demonstrate that this structure is a Barr Body?

11. Figure 3 shows that the authors checked 5 different genes to examine silencing of the entire chromosome. Why is this necessary? What is the result?

12. In Figure 3 panel a, the 5 day and 20 day FISH experiments show a very large spot red corresponding to Xist on Chromosome 21. Why is this spot so much larger than the spots for the other gene (APP)? What does this large spot indicate?

For the next several questions you will need to examine Figure 4 - this is a difficult figure, but is an important control for the silencing of the trisomic Chr 21.

13. The authors want to compare the change in Chr21 gene expression in the same Trisomy 21 cell before and after activation of Xist (this is "+/- Dox" as the Xist gene is turned on by the addition of doxycycline), to the change in chr21 gene expression between a Trisomy 21 cell and a normal (disomic) cell. Why is this an important comparison to make? (HINT: think back to the intro - what is the point of doing this study?)

14. Next, the authors then compare normal disomic and corrected trisomic cells - why is this an important comparison to make?

15. What is a CpG island? What change in gene expression occurs when a CpG island is methylated?

16. The authors examine CpG methylation on the silenced Chr21 as well as in other chromosomes (Figure 4b). What do they find? Is this expected, and does it make sense?

17. The authors finally hypothesize that trisomy 21 could affect cell growth and/or the differentiation of stem cells into neurons. Therefore, reduction in Chr21 gene expression could improve these growth defects. Examine Figure 5. Is their hypothesis correct? What specifically did they measure, and what differences did they see between the trisomic cell and the corrected cells?

18. Under the section entitled "Towards future applications" the authors include descriptions of several important control experiments that are not shown in the main article. One such experiment involves the

withdrawal of doxycycline. What is the point of this statement? Why is it desirable to be able to withdraw doxycycline?

19. The final sentence of the paragraph under “Toward future applications” notes that this method of Chr21 silencing is compatible with normal X chromosome silencing. What do you suppose they mean by that? Why is this a very important point if this technology is to be used as a therapy in the future?

20. Make note of the two or three reasons why the authors believe this research is an important step in the future treatment of human disease.