Annotated Bibliography

1. Barakat, T. S., & Gribnau, J. (2014). Combined DNA-RNA fluorescent in situ hybridization (FISH) to study X chromosome inactivation in differentiated female mouse embryonic stem cells. *Journal of visualized experiments: JoVE*, (88).

Before doing this project, I did not know how to perform an RNA/DNA FISH experiment. This paper reviews the RNA/DNA FISH protocol and helped me understand how RNA/DNA FISH experiments work.

The authors of this paper give a detailed step-by-step RNA/DNA FISH protocol and give useful tips on increasing the efficiency of the experiment.

2. Barlow, D. P., Stöger, R., Herrmann, B. G., Saito, K., & Schweifer, N. (1991). The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature*, *349*(6304), 84-87.

In this paper, authors studied allele specific expression of *Igf2r*, *Sod2* and *Tcp-1* genes. They found that in embryonic day 15 (E15) embryos, *Igf2r* expression is monoallelic whereas *Tcp-1* and *Sod2* expression is biallelic.

This paper helped me develop my research question. Before I read this paper I knew I wanted to study the functional role of *Airn* RNA in the cell after *Igf2r* acquires a DNA methylation mark but I did not know what target genes to study.

Sod2 and *Tcp-1* are closely linked to *Igf2r* and all these genes are located near *Airn*. Thus I reasoned that *Airn* could interact with these genes later in development and in newborn/adult mice.

3. Hiroshi, K., Keith, W., Alan, A., Masami, N., Hiroshi, M., Hideyuki, Y., Matsushiro, A. & Takashi, M. (1992). Structure and expression of the gene encoding mouse t-complex polypeptide (Tcp-1). *Gene*, *120*(2), 207-215.

Authors of this paper studied *Tcp-1* expression in testis, thymus, heart, brain and lung tissue from mice. They found that *Tcp-1* is expressed in all the tissues studied.

This study helped me design my experiment and choose which tissue types to examine to determine if *Tcp-1* is an imprinted gene in mice.

4. Hu, J. F., Balaguru, K. A., Ivaturi, R. D., Oruganti, H., Li, T., Nguyen, B. T., Vu, T. H. & Hoffman, A. R. (1999). Lack of reciprocal genomic imprinting of sense and antisense RNA of mouse insulin-like growth factor II receptor in the central nervous system. *Biochemical and biophysical research communications*, *257*(2), 604-608.

Hu *et al.* examined the expression of *Airn* and *Igf2r* in newborn and adult tissue. In newborn mice, they studied heart, brain, liver, kidney and lung tissue and in one-month-old mice they studied heart, lung, liver, spleen, kidney and intestinal tissue.

Although *Airn* and *Igf2r* are expressed in all the tissue types, they found that the expression pattern of *Igf2r* changes in brain tissue. In newborn mice, *Igf2r* expression is monoallelic and in one-month-old mice its expression is biallelic.

This paper demonstrates that the imprint status of genes can change over time in a tissue specific manner thus it is possible that *Tcp-1* and *Sod2* are imprinted genes however, when Barlow *et al.* studied their expression, it was at a point when they were not imprinted.

Lastly, I used this paper to fine tune my experimental design. I wanted to study tissue types where I knew *Airn* was expressed and this paper demonstrates that *Airn* is expressed in heart, brain, liver, kidney and lung tissue in newborn mice.

5. Jones, P. L., Kucera, G., Gordon, H., & Boss, J. M. (1995). Cloning and characterization of the murine manganous superoxide dismutase-encoding gene. *Gene*, *153*(2), 155-161.

Authors of this paper studied *Sod2* expression in heart, spleen, thymus, lung, kidney, heart, testis and brain in mouse tissue. They found that *Sod2* is expressed in all tissues studied.

This study helped me design my experiment and choose which tissue types to examine to determine if *Sod2* is an imprinted gene in mice.

6. Lee, J. T., & Bartolomei, M. S. (2013). X-inactivation, imprinting, and long noncoding RNAs in health and disease. *Cell*, *152*(6), 1308-1323.

This is a review paper that discusses the role of genomic imprinting in disease development. This paper highlights the importance of understanding the role of imprinted long non-coding RNAs (lncRNAs) in order to treat disease.

7. Nagano, T., Mitchell, J. A., Sanz, L. A., Pauler, F. M., Ferguson-Smith, A. C., Feil, R., & Fraser, P. (2008). The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science*, *322*(5908), 1717-1720.

This study looked at *Airn* RNA associating with the *Slc22a3* locus in mouse placentas.

Nagano *et al.* performed an RNA/DNA FISH and an RNA TRAP in order to study the interaction between *Airn* RNA and the *Slc22a3* locus.

The authors found that Airn forms a cloud around the paternal *Slc22a3* locus.

This study helped me design the experiment for my final project. *Airn* RNA acts in cis to regulate *Slc22a3* expression. This study helped me fine tune my research question: if *Airn* RNA interacts with *Slc22a3* and forms a cloud around the *Slc22a3* locus then *Airn* RNA might form clouds around other loci that it interacts with in cis. Using an RNA/DNA FISH experiment I can study the localization pattern of *Airn* relative to *Tcp-1* and *Sod2* and can infer whether *Airn* is associating with these loci.

8. Santoro, F., Mayer, D., Klement, R. M., Warczok, K. E., Stukalov, A., Barlow, D. P., & Pauler, F. M. (2013). Imprinted Igf2r silencing depends on continuous Airn lncRNA expression and is not restricted to a developmental window. *Development*, *140*(6), 1184-1195.

In this study, researchers demonstrated that continuous *Airn* expression is necessary and sufficient to silence *Igf2r* expression in ES cells but only until DNA methylation is established.

Authors also demonstrated that Airn expression is not necessary to maintain DNA methylation of *Igf2r*.

This paper was very important for the development of my research project because it helped me develop my research question.

It was through reading this paper that I realized that there is a gap in our understanding of *Airn* expression. We do not yet understand why *Airn* is continuously expressed once DNA methylation of *Igf2r* is established.

9. Wang, K. C., & Chang, H. Y. (2011). Molecular mechanisms of long noncoding RNAs. *Molecular cell*, *43*(6), 904-914.

This was one of the first papers I read when I started researching my final project. I knew I wanted to study lncRNAs but I did not have a research topic yet. In this review article, the authors discuss the role of lncRNA in imprinted gene expression and look at several imprinted lncRNAS in the cell.

Airn is one of the lncRNAs the authors review. I became interested in *Airn* because it is unique in that it represses *Igf2r* expression in a transcription-dependent manner. For this reason I decided I wanted to study *Airn* and its function in the cell for my final project.

10. Zwart, R., Sleutels, F., Wutz, A., Schinkel, A. H., & Barlow, D. P. (2001). Bidirectional action of the Igf2r imprint control element on upstream and downstream imprinted genes. *Genes & development*, *15*(18), 2361-2366.

Although this paper is not a review, in the introduction section the authors provide extensive information on imprinting. Authors of this paper review the molecular mechanism behind imprinting.

One of the reasons I chose my research topic is because I wanted to learn more about imprinting. This paper helped me understand how imprinting works and was helpful for when I wrote the background section of my final project.