FINAL PROJECT OUTLINE

Student's name: Leah T'ien

Topic chosen: For my final project I am going to study the imprinted *Airn* gene and the role of *Airn* RNA in newborn mice.

Specific Question: Does *Airn* RNA associate with either the *Tcp-1* or *Sod2* loci?

How is this question novel and original? We know that *Airn* expression is necessary and sufficient for *Igf2r* repression up until *Igf2r* acquires a DNA methylation mark (Santoro et al., 2013). Methylation of the *Igf2r* genes causes it to be stably repressed. Although we expect that after *Igf2r* methylation, *Airn* expression should halt what we see is continuous *Airn* expression throughout development and expressed in adult tissues as well (Hu et al., 1999). Therefore, this question is novel and original because we do not understand why *Airn* is expressed once *Igf2r* acquires a DNA methylation mark because as far as we know *Airn* does not have other regulatory roles in the cell. My research proposal seeks to identify a functional role for Airn in newborn mice in order to understand why *Airn* is expressed in mature tissues.

Potential impact of the proposed question (were it to be answered by your proposed experiment): My proposed question would have a big impact on the medical and developmental biology community because imprinted genes are important during development and aberrant expression of these genes is associated with serious congenital and X-inactivation diseases (Lee & Bartolomei, 2013). Furthering our understanding of the processes that imprinted genes are involved in can shed light on the function of other imprinted genes in the cell and can also help us design better therapies to treat diseases.

Hypothesis: I hypothesize that *Airn* RNA is localized at the *Tcp-1* and *Sod2* loci in newborn mice heart, brain and lung tissue.

Evidence on which the hypothesis is based: If *Airn* is regulating the expression of genes in newborn it is most likely acting in cis because *Airn* encodes an unstable lncRNA that has a short half life (Santoro et al., 2013). We know that *Igf2r* is an imprinted gene whose regulation is governed by *Airn* expression (Santoro et al., 2013). *Igf2r* is closely linked to *Tcp-1* and *Sod2* gene loci - all three genes are located on chromosome 17 and are located near the *Airn* loci (Barlow et al., 1991). We know *Airn*, *Tcp-1* and *Sod2* are all expressed in heart, brain and lung tissue therefore it is possible that in all these tissues *Airn* is localized at the *Tcp-1* and *Sod2* loci in order to regulate their expression (Hiroshi et al., 1992; Jones, Kucera, Gordon & Boss, 1995; Hu et al., 1999).

Predictions (s): I predict that Airn will be localized at the Tcp-1 and Sod2 loci in heart, brain and lung newborn mice tissue.

Experimental approach to test to test prediction (include any details that you have worked out so far: In order to test my hypothesis I am going to perform an RNA/DNA FISH experiment. RNA/DNA FISH experiments allow you to study the localization pattern of an RNA transcript relative to a genomic sequence. Therefore, RNA/DNA FISH will allow me to determine whether *Airn* RNA is localized at the *Tcp-1* and *Sod2* loci.

List of relevant primary and review articles read, and summary of relevant information from each (this is the start of the annotated bibliography that you will need to include in your portfolio):

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

This paper discusses the importance of imprinting research and studying the role of lncRNAs in the cell.

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.

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.

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.

Potential ways to make your question known to the public at large (e.g. to your non-biologist family and friends): One of the ways I could make my question known to the public at large is by writing a small piece for the local newspaper or by being a guest on a radio/podcast episode. I think if I highlighted the potential impacts of imprinting research, then the public would be interested in learning about my study and how it has the potential to change the way we think about imprinted genes.

Any other parts of the project completed so far:

Anything you would like specific feedback on: