HOTAIR in mice - circa 2011

## Questions

1. Please list the names of all the group members who participated:

Alice Wang, Buffy Chen

1. What prompted the authors to conduct the study that they report in the paper? What did they know before they started?

They conducted the study because long non-coding RNAs are important for regulation and in early human development. They knew that in humans, the HOTAIR regulates HOXD genes in trans via the recruitment of PRC2. As mice is an important model for early development, it was important for the researchers to study HOTAIR in mouse as well.

1. What was the overall experimental process that the authors followed as they conducted this study (this can be represented as a simple flowchart)

-looked for the presence of Hotair in the mouse genome

-pair-wise sequence alignment with the mouse DNA segment, using the rVISTA software -transcriptome analyses

-look at expression of mHotair by in situ hybridization (WISH) on developing mouse embryos

-look at the expression of these potential target genes in the absence of mHotair

-did quantitative RT-PCR analyses on the forebody, hindbody, forelimbs and hindlimbs of HoxCDel/Del embryos

-in situ hybridization on mutant animals to find distribution of Hoxd10 transcripts (main target of hHotair)

-chromatin immunoprecipitation (ChIP) on mouse embryos then quantitative RT-PCR (check H3K27me3 amount)

1. As a group, decide which figure is the most important in this paper (one good criterion to use is to think about what the most important point of the paper is, and which figure contributes the most to making this point).

Figure 2

1. For your selected figure, please answer the following questions:
   * 1. What was the experiment that lead to the results? (What were the authors asking, what did they do, what did they measure, what were the controls).

They looked at the expression of the potential target genes in the absence of mHotair. They used a full deletion of the HoxCluster where all the HoxC cluster nearby were deleted. They isolated the HoxC del/del embryo at embryonic day at 13.5 and dissected it into four distinct pieces: forebody, hindbody, forelimbs, and hindlimbs. They followed this up with RT-PCR analyses on the various samples using wild-type and heterozygous littermate as controls for homozygous mutant samples.

* + 1. What do the data show?

The data showed that mHotair was detected neither in Hox C del/del mutant embryos, nor in forebody samples of all three genotypes, which was used as negative controls. In the other samples, mHotair scored very low. There were no differences noted in the expression level of the presumptive targets.

* + 1. What can we conclude from the data?

It is not necessary for mHotair to perform any regulatory activity in trans over Hoxd cluster genes in mice.

1. What did you learn about lncRNAs (or linc RNAs) while reading this paper? It is OK to just provide a list of facts and/or ideas that group members learned.

## -lincRNAs can act in trans to regulate the expression of other genes

-the mechanism performed by lncRNA is not necessarily conserved across species