Class 22: CRISPR-Cas9 genome engineering

Reading assignment

- Smith et al. A unique regulatory phase of DNA methylation in the early mammalian embryo. 2012 Nature:339-344.

Classroom activity (limit 45 minutes)

1. What is a "CpG island"? What form of methylation can be present in CpG islands? What effect does methylation, or lack of it, of CpG islands have on the transcription of nearby genes?

   CpG islands are regions that have many DNA sequences of 5'-CG-3'. They are commonly found in the promoter regions upstream of structural genes. The C of these sequences can be methylated to 5-methylcytosine (5-meC). Methylation is associated with lack of transcription and non-methylation with active transcription of the gene.

2. Smith et al. used "reduced representation bisulfite sequencing" (RRBS) to identify nucleotides in the mouse genome that are methylated. How is methylation of CpG islands recognized by exposing DNA to bisulfite?

   Reduced representation bisulfite sequencing targets regions that contain CpG sequences by the DNA being cleaved with a restriction enzyme MspI that cuts 5'-CCGG-3', which includes a CpG site, and then selecting small sized DNAs (hundreds of nucleotides). These are then sequenced by next generation sequencing after treating the DNA with bisulfite, which modifies C to U but has no effect on 5-meC. By comparing the sequence of bisulfite treated DNA to untreated DNA you can identify each C that is changed to U (non-methylated) and each unchanged (5-meC).

3. What are the general pattern of demethylation and methylation of the maternal and paternal chromosomes during early development? How does the embryonic methylation pattern differ from that seen in somatic tissues in the adult? What purpose do you think this serves for the differentiation process?

   Oocytes are globally less methylated than sperm but the zygote paternal DNA becomes largely demethylated. In both sperm and somatic cells methylation of CpG is inversely related to the density of the sites, so CpG islands are less methylated in general than the CpGs in less dense regions. Methylation returns as development progresses until the preimplantation embryo which is highly methylated similar to somatic cells and sperm. Demethylation in the oocyte is thought to allow expression of early embryonic genes that are silenced by methylation in somatic cells.

4. When did Smith e al. find the most dramatic changes in methylation occur? How did they show that? What is happening in the embryo during those stages? Why do you think that changes in methylation might happen at those specific stages?

   The two changes are from sperm to zygote (substantial demethylation) and from the early inner cell mass (ICM) to the preimplantation embryo (substantial remethylation). The first changes occurs as development is beginning and the second after the early stages of development are complete. The demethylation allows expression of the early developmental stage genes and remethylation represses their expression after they are no longer needed to be expressed.

5. What do the authors mean when they say that "the oocyte defines the early methylation landscape?" What data in the Smith et al. article support that conclusion? Is it always the case that the methylation state of the embryonic DNA is similar to that of the DNA in the oocyte? How do the researchers show that?

   The methylation state of the oocyte and the early embryo are substantially identical. Methylation of the paternal chromosomes is reduced to make them similar to the maternal chromosomes, resulting in an early embryo that resembles the oocyte. The bisulfite sequencing shows a similar distribution of CpG methylation and non-methylation in the two stages. Hypermethylated regions of the oocyte DNA, however, actually become less methylated in the zygote, consistent with a trend toward reduced methylation overall.

6. What is a "retroelement"? These elements are usually thought of as being inessential to the host—parasitic DNAs that are carried in the genome but provide no function necessary to the mouse. What evidence do Smith et al. quote that suggests that this is not the case? How might this evidence explain the purpose of the demethylation of the LINE family of retroransposons during a specific stage of development?

   A retroelement is a transposon in the mouse genome that replicates by an RNA intermediate, similar to a retrovirus but does not have an extracellular particle stage but rather the copies move to a new chromosomal location. These elements tend to be less methylated in oocyte than in sperm DNA and both maternal and paternal elements become less methylated in the zygote. They quote evidence that expression of the L1Md_T class of elements are required for embryos to progress through cleavage, suggesting that they may modulate the activity of genes important at that stage.

7. Some paternal and maternal methylation patters are maintained from the gametes throughout embryogenesis and into the adult somatic tissue. Where did the researchers find that the oocyte and sperm DMRs were located in the mouse genome? What effect do you think the epigenetic inheritance of these differentially methylated regions (DMRs) would have on adult gene expression?

   The oocyte DMRs tend to be in genes and their promoters while the sperm DMRs tend to be in intergenic regions. The purpose of the DMRs appears to be to control maternal vs paternal chromosome transcription.