In class assignment: Pasque and Plath (2015) review paper

In collaboration with one or two classmates, discuss the following and record your answers to five of the following questions (point form is fine).

5 chosen questions:

**What are the main differences between mouse ES cells and human iPS cells in terms of XCI?**

-          mESCs: naïve stated (Xa, Xa). They undergo two rounds of XCI, and one round of XCR.

-          hiPSCs: primed stated (Xa, Xi). One round of XCI. In time, Xi will erode to Xe.

**What does what we know about XC reactivation (XCR) suggest about the roles of pluripotency factors in maintaining pluripotency and preventing/hindering cell determination?**

XCR is induced by sequential activation of pluripotency factors.

Pluripotency factor Nanog is activated early in the process. Nanog and Tsix repress Xist. Subsequently, genes on the Xi along with other pluripotency factors are reactivated. This seems partially Nanog-dependent, since Nanog knockouts/knockdowns have decreased XCR, and decreased iPSC formation (but still have the ability).

No single factor is sufficient for XCR.

This suggests that the pluripotency factors induce/maintain pluripotency by upregulating pluripotency associated genes, some of which reside on the X chromosome. This happens through chromatin alterations and e.g. Xist activity.

**The authors describe a lot of observations they (and others) made about XCR (see for example the second column on page 77). Some include causal relationships, but many are purely descriptive. Select one “step” of XCR described there and propose an experiment to investigate cause-effect relationships between two factors.**

The authors of the paper suggest that recruitment of EZH2 to the Xi during reprogramming

is not required for XCR, but is an intermediate stage in which cells are in a dedifferentiated state that precedes pluripotency. To test for this, we could knockout EZH2 by site directed mutagenesis and look for reactivation of XCR to support this statement. If Xi would remain inactive we could conclude that EZH2 is necessary for XCR.

**What is the role of *Tsix* in mouse, and in mouse XCR?**

Tsix is involved in repression of Xist and hindrance of Xist induction on the active X chromosome. Xist is required for XCI and involved in maintenance of Xi, therefore Tsix is important to XCR by repressing Xist. It is shown that Tsix may be required for Xist repression, but the repression of Xist is not sufficient for XCR, other factors plays a role such as DNA methylation.

**What is Xi erosion? In what cells does it happen?**

Xi erosion is the epigenetic alteration of the Xi to an Xe.

Characteristics:

* Loss of XIST
* H3K27me3 foci (changes in epigenetic marks)
* Loss of promotor DNA methylation (loss of transcriptional repression)
* Trancriptional (re)activation of XACT (and other genes)

It happens in hiPSCs that are cultivated without differentiation.

hiPSCs are thought to be epigenetically instable.

Loss of XIST has been correlated with upregulation of X-linked oncogenes.