Requirement of a novel dose-dependent X-linked factor in X-chromosome inactivation

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The disparity in X-chromosomal dosage between male and female mammals is remedied via transcriptional inactivation of most genes along one of the two X-chromosomes in females, in a process referred to as X-chromosome inactivation. Once inactivated early in embryogenesis, replicated copies of that X-chromosome are transmitted as inactive through many rounds of cell division, essentially for the life of the individual. X-inactivation therefore serves as a paradigm of epigenetic regulation. Conventionally, X-inactivation is thought to initiate via the upregulation of the Xist long non-coding RNA (lncRNA). Xist is transcribed only from the inactive-X and physically coats the chromosome in *cis*. In so doing, Xist recruits protein complexes that are posited to epigenetically silence gene expression. Here we analyze the sufficiency of Xist RNA coating to cause X-inactivation in male and female stem cells. We find that Xist induction in male mouse epiblast stem cells (EpiSCs) and embryonic stem cells (ESCs) is innocuous: in a vast majority of cells, Xist RNA coating does not lead to X-inactivation in males. On the other hand, in female EpiSCs and ESCs, Xist RNA induction almost always coincides with silencing of genes in *cis*. We also confirm this difference in X-inactivation states between males and females in mouse embryos that ectopically express Xist. Together, our *in vitro* and *in vivo* results therefore show that Xist lncRNA is insufficient to trigger X-inactivation in males, and implicate a sex-specific activity in triggering X-inactivation in females. We propose that a novel X-chromosomal factor that escapes X-inactivation is required to initiate X-inactivation in females. Its increased dosage in XX females relative to XY males triggers Xist induction and X-inactivation only in females, and is also the means by which the cell 'counts' its complement of X-chromosomes prior to inactivating one. By profiling EpiSCs via allele-specific RNA-Seq, we have compiled a short list of candidate X-inactivation escapees required to trigger Xist and X-inactivation. We will present data testing the role of these candidates in X-inactivation.