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New and Xisting regulatory mechanisms of X chromosome inactivation

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Equalization of X linked gene expression is necessary in mammalian cells due to the presence of two X chromosomes in females and one in males. To achieve this, all female cells inactivate one of the two X chromosomes during development. This process, termed X chromosome inactivation (XCI), is a quintessential epigenetic phenomenon and involves a complex interplay between noncoding RNAs and protein factors. Progress in this area of study has consequently resulted in new approaches to study epigenetics and regulatory RNA function. Here we will discuss recent developments in the field that have advanced our understanding of XCI and its regulatory mechanisms.

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Introduction

X chromosome inactivation (XCI) evolved in mammals to balance X-linked gene expression levels between males (XY) and females (XX) [1–4]. During development females undergo two forms of XCI: Imprinted and Random. Imprinted X inactivation is encountered during early embryogenesis, where the paternal X chromosome (Xp) is preferentially silenced. While this state is maintained in extra-embryonic tissues throughout development, all imprinted epigenetic marks on Xp are erased in cells of epiblast lineage, which will form the future embryo-proper, to initiate another round of XCI where either Xp or the maternal X chromosome (Xm) is silenced randomly (random XCI) [5].

Mouse embryonic stem (mES) cells are derived from the epiblast of early embryos in which both X chromosomes are active. mES cells offer a tractable system to study random XCI because they recapitulate this process upon differentiation *in vitro* and can be genetically manipulated. In the undifferentiated state, each cell contains two active X chromosomes (Xa). Upon differentiation each cell first counts the number of X chromosomes within the cell and then randomly chooses to inactivate one X chromosome. After a choice is made, Xist RNA is upregulated on the future inactive X (Xi) and a gradual chromosome wide silencing process is initiated. Once established, this silent state is transmitted through each round of cell division in a stable and heritable manner.

In the past few years, substantial progress has been made in understanding the regulation of XCI. Novel roles for long noncoding (lnc) RNAs as well as their interplay with various protein factors have been identified [6•], revealing detailed mechanisms involved in this process (Figure 1). Links have been uncovered connecting XCI to the pluripotency network. In this review, we will discuss recent advances in the field with an emphasis on regulatory RNAs and protein factors involved in X chromosome silencing.

New insights into lncRNA regulation of XCI

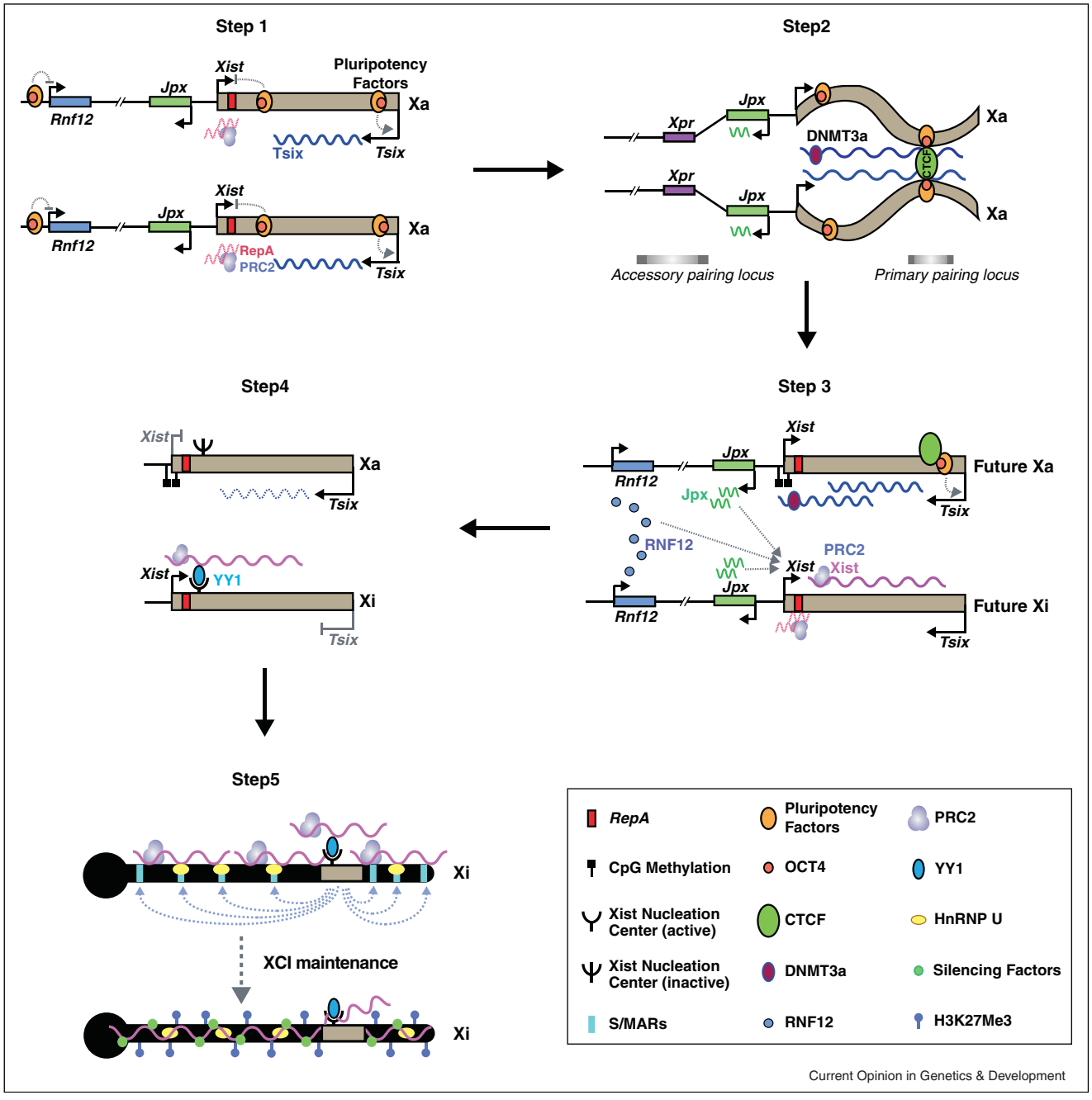
Xist

Xist is a 17 kb RNA that is transcribed exclusively from Xi and coats it in *cis* [7–10]. It is comprised of several repetitive units, Repeats A–F. *Repeat A* is located at the 5′ end of *Xist* and is the region conferring silencing ability to this RNA [11]. The motif within Xist RNA is now known to recruit Polycomb Repressive Complex 2 (PRC2) to the Xi [12]. PRC2 in turn catalyzes histone H3 Lysine 27 trimethylation (H3K27me3), a mark associated with repressed chromatin. Subsequent events involved in the maintenance of Xi include incorporation of the histone variant MacroH2A and DNA methylation [1]. As Xist is a key molecule that triggers chromosome-wide silencing, much effort has been devoted to understanding how *Xist* is regulated. In addition to transcription factors and *cis*-elements within the *Xist* locus, many regulatory factors are lncRNAs or loci encoding them. Like transcription factors, RNA regulators of *Xist* come in two flavors: activators and repressors (Figure 2a). In the next subsections, we discuss recent developments in this arena.

RepA, a Repeat A RNA

Previous transgenic studies showed that the Repeat A region of *Xist* is required for silencing in ES cells, as deleting Repeat A on a transgene precluded Xist from silencing genes *in cis* [11]. When a similar deletion was introduced into mice, the X-chromosome bearing the deletion could no longer be silenced. Interestingly, this

Figure 1



Random X chromosome inactivation in mouse embryonic stem cells upon differentiation. Step 1 — In undifferentiated ES cells, OCT4 and other pluripotency factors repress expression of *Xist* and the *Xist* activator *Rnf12*. Pluripotency factors also directly activate *Tsix* expression. *Tsix* is expressed from both X chromosomes and prevents activation of *Xist* in *cis*. Step 2 — At the onset of differentiation, X chromosomes pair via interaction between OCT4 and CTCF resulting in uneven distribution of transcription/pluripotency factors on the two Xs. This leads to simultaneous activation of Xist from the future Xi at the same time repressing *Tsix* from the same allele. Tsix recruits the DNA methyltransferase DNMT3a which methylates *Xist* promoter thereby silencing it on the future Xa. Step 3 — RepA RNA is expressed from the future Xi and recruits the PRC2 complex. *Jpx* and *Rnf12* are upregulated from both the Xi and Xa. A combination of all three events leads to activation of *Xist* expression. Xist in turn recruits PRC2 to the Xi. Step 4 — YY1 tethers Xist to the Xi and mediates spreading in *cis* along the entire Xi. Step 5 — Xist tethered onto Xi spreads via hnRNP U and S/MAR sites. Eventually, Xist coats the entire Xi and recruits various silencing factors to maintain Xi in a repressed state.