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Concise Review: Pluripotency and the Transcriptional Inactivation of the Female Mammalian X Chromosome

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ABSTRACT

X chromosome inactivation (XCI) is a striking example of developmentally regulated, wide-range heterochromatin formation that is initiated during early embryonic development. XCI is a mechanism of dosage compensation unique to placental mammals whereby one X chromosome in every diploid cell of the female organism is transcriptionally silenced to equalize X-linked gene levels to XY males. In the embryo, XCI is random with respect to whether the maternal or paternal X chromosome is inactivated and is established in epiblast cells on implantation of the blastocyst. Conveniently, ex vivo differentiation of mouse embryonic stem cells recapitulates random XCI and permits mechanistic dissection of this stepwise process that leads to stable epigenetic silencing. Here, we focus on recent studies in mouse models characterizing the molecular players of this female-specific process with an emphasis on those relevant to the pluripotent state. Further, we will summarize advances characterizing XCI states in human pluripotent cells, where surprising differences from the mouse process may have far-reaching implications for human pluripotent cell biology. STEM CELLS *2012;30:48–54*

Disclosure of potential conflicts of interest is found at the end of this article.

THE NONCODING RNA *XIST* CONTROLS THE INITIATION OF RANDOM XCI

The importance of X chromosome inactivation (XCI) is demonstrated by the fact that ablation of the master regulator of this process, *Xist* (X-inactive specific transcript), leads to female-specific lethality early in embryonic development in mice [1, 2]. The X-linked Xist gene encodes an approximately 17 kb spliced and polyadenylated transcript that is essential for heterochromatin formation on the X chromosome from which it is transcribed [1–4]. In the embryo, XCI is random based on the parent-of-origin for the inactive X (X_i), such that female organisms are mosaic for which X chromosome is expressed. In vivo, random XCI is initiated in epiblast cells of the inner cell mass (ICM) of the blastocyst soon after implantation and, in vitro, on induction of differentiation in mouse embryonic stem cells (mESCs), which are derived from epiblast cells of the preimplantation blastocyst. On initiation of XCI, *Xist* is transcriptionally upregulated on the future X_i [5, 6]. It has been suggested that the transcription factor Yin-Yang 1 (YY1) tethers *Xist* RNA to its site of transcription by binding directly to both *Xist* RNA and DNA [7]. The RNA then spreads and creates an "*Xist* RNA cloud" demarcating the nuclear domain of the inactivating X; however, the regulation of the release of *Xist* RNA from the Yy1 tether at the site of transcription is still unknown.

As *Xist* RNA molecules coat the X, they trigger transcriptional silencing with immediate exclusion of RNA polymerase II [8]. This is followed by loss of active chromatin marks and establishment of silencing chromatin marks, which occur in an ordered sequence of events and include, for example, trimethylation of histone H3 lysine 27 ($H3K27me^3$) by the Polycomb complex PRC2, DNA methylation of promoter regions, and recruitment of the repressive histone variant macroH2A [9]. The result is that the X_i is maintained late replicating in S phase through the lifetime of the organism. *Xist* transcription and coating of the X_i continues in somatic cells, with *Xist* RNA dissociating from the X_i in mitosis and recoating the X in early G_1 of the cell cycle [10]. Although *Xist* depletion during initiation of XCI leads to reversal of X chromosome silencing and heterochromatin formation, its deletion in somatic cells has only minor effects on X_i reactivation as the RNA acts synergistically with other repressive chromatin modifications that accumulate on the X_i during differentiation [11, 12].

Transcription and spreading of *Xist* RNA along the X is a prerequisite for silencing, which is not X-restricted as

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silencing can spread across X:autosome translocations, and transgenic *Xist* can induce silencing of neighboring autosomal DNA [12]. The spread of *Xist* RNA-mediated silencing into autosomal regions is variable and has been proposed to correlate with the density of retrotransposons belonging to the family of long interspersed elements (L1) [13]. A recent report suggested that the silencing of X-linked L1s occurs prior to X-linked gene silencing and may promote the nucleation of heterochromatin. Conversely, specifically a subset of young L1 elements becomes transcribed on *Xist* RNA coating and may help the local propagation of XCI [14]. In support of a functional role for L1 elements in XCI, the human X chromosome has a twofold enrichment in L1 elements relative to autosomes [15]. Still it remains to be seen whether the behavior of these repetitive elements is a functionally important means of *Xist*-dependent facultative heterochromatin formation. In the following sections of this review, we will discuss how *Xist* is regulated in pluripotent cells of the mouse.

Acquisition of Pluripotency in Mice Is Coupled to X_i Reactivation

In the mouse, XCI occurs in two forms that differ in parent-of-origin effect and in the developmental timing of initiation. Imprinted XCI, where the paternal X chromosome (X_p) is inactivated, is established in the mouse preimplantation embryo at the four-cell stage and occurs in all cells of the preimplantation embryo (Fig. 1) [16–21]. As the mid-blastocyst stage is reached (prior to implantation), imprinted XCI is reversed only in the subset of cells in the ICM that give rise to the epiblast, so that the cells that form the future embryo carry two active X chromosomes (X_aX_a) without *Xist* RNA coating [16, 18, 21, 22] (Fig. 1). Reactivation of the X_p is a prerequisite for subsequent random XCI in the epiblast on implantation of the blastocyst [16, 18]. In contrast, the imprinted form of XCI is maintained in the extraembryonic tissues.

Random and imprinted XCI differ in the molecular requirements for initiation and reactivation. In vivo evidence shows that, although *Xist* RNA coats the X_p, it is not required when imprinted XCI first occurs at the four-cell stage (as it is for random XCI). Rather, *Xist* RNA coating is needed to complete and stabilize the silencing of the imprinted X_i [17, 19, 20]. With respect to X_i reactivation, a recent study demonstrates that the reactivation of the imprinted X_p occurs in two steps, with induction of biallelic expression of X-linked genes preceding the disappearance of *Xist* RNA coating, in agreement with the notion that *Xist* RNA coating and silencing of the X_p are uncoupled at this point in development [21]. The mechanisms that lead to gene activation on the X_p and *Xist* silencing are still unclear but linked to the specification of the epiblast lineage, as preimplantation embryos lacking the pluripotency transcription factor Nanog are unable to specify the epiblast lineage and do not induce the loss of *Xist* RNA coating and Polycomb protein enrichment on the X_i [22]. Nanog appears to be directly involved in the regulation of *Xist* because preimplantation embryos with a genetically engineered overexpression of Nanog lose *Xist* RNA more rapidly, although without affecting the timing of X_p reactivation [21]. However, Nanog may not be sufficient for this effect on *Xist* as Nanog is already present in the X_i-bearing cells of the late morula and becomes restricted as the pluripotent X_aX_a epiblast lineage forms, indicating that other epiblast-linked mechanisms must synergize with Nanog to control *Xist* repression [21, 22].

It is now appreciated that X chromosome reactivation (XCR) also occurs during the experimentally induced acquisition of pluripotency through either transcription factor-induced reprogramming to induced pluripotent stem cells (iPSCs), somatic cell nuclear transfer or ESC/somatic cell fusion [23–25]. XCR during reprogramming of mouse somatic cells to iPSCs leads to loss of heterochromatic marks of the X_i and *Xist* repression, such that random XCI is observed on differentiation of mouse iPSCs (miPSCs), as in mESCs [23] (Fig. 1). It has been demonstrated that XCR is a late event in miPSC reprogramming, occurring at around the time of pluripotency gene activation [26], but insight into the mechanism and the events leading to X_i reactivation is still lacking. Nevertheless, the establishment of pluripotency both in vitro via reprogramming and in vivo during the establishment of the epiblast lineage in preimplantation embryos is coupled to XCR and *Xist* repression. Therefore, the X_aX_a state is a key attribute of the pluripotent state of mESCs and miPSCs.

Importantly, studies with a doxycycline-inducible *Xist* transgene have shown that *Xist*-dependent gene silencing is possible in undifferentiated male and female mESCs but no longer after induction of differentiation or in somatic cells [12]. This observation illustrates that *Xist* function is context-dependent but not with respect to sex, as factors required for the silencing process are present in male and female undifferentiated mESCs. As the active state of the X chromosomes must therefore be ensured by strong transcriptional repression of *Xist* in mESCs, one can view initiation of XCI on differentiation of mESCs from the perspective of loss of *Xist* repression.

Xist Is Regulated by Its Antisense Transcript *Tsix*

A major antagonizing factor to *Xist* in mESCs is another long noncoding RNA, *Tsix*, transcribed antisense to *Xist* specifically in mESCs and downregulated first on the X_i and then on the X_a during differentiation [27] (Fig. 2). Loss of *Tsix* function on one of the two female Xs leads to slight upregulation of *Xist* transcript levels in undifferentiated mESCs and skewing of XCI toward the *Tsix*-deleted X on differentiation [28, 29]. These observations suggest that *Tsix* mainly regulates the monoallelic induction of *Xist* in the choice aspect of XCI. In support of this idea, live-cell imaging of differentiating female ESCs carrying X chromosomes tagged with a tetO array bound by a tetR-mCherry fusion confirmed a previously shown transient pairing of homologous *Xist/Tsix* regions of the two X chromosomes and demonstrated that this interaction is associated with exclusive deafening of the *Tsix* allele on the future X_i, which is proposed to allow upregulation of *Xist* [30–32]. *Tsix* antagonism of *Xist* requires transcription through the *Xist* locus and the mechanism is suggested to involve change in the chromatin structure around the *Xist* 5′ regulatory region [33, 34]. Together, these findings indicate that *Tsix* is not the only repressor of *Xist* in pluripotency but also other factors must be involved in keeping *Xist* downregulated (Fig. 2).

Pluripotency Transcription Factors Directly Repress XCI in Escs

Oct4, Sox2, and Nanog form a transcription factor triad that is key in maintaining ESC identity by activating genes of the self-renewal program and repressing lineage commitment

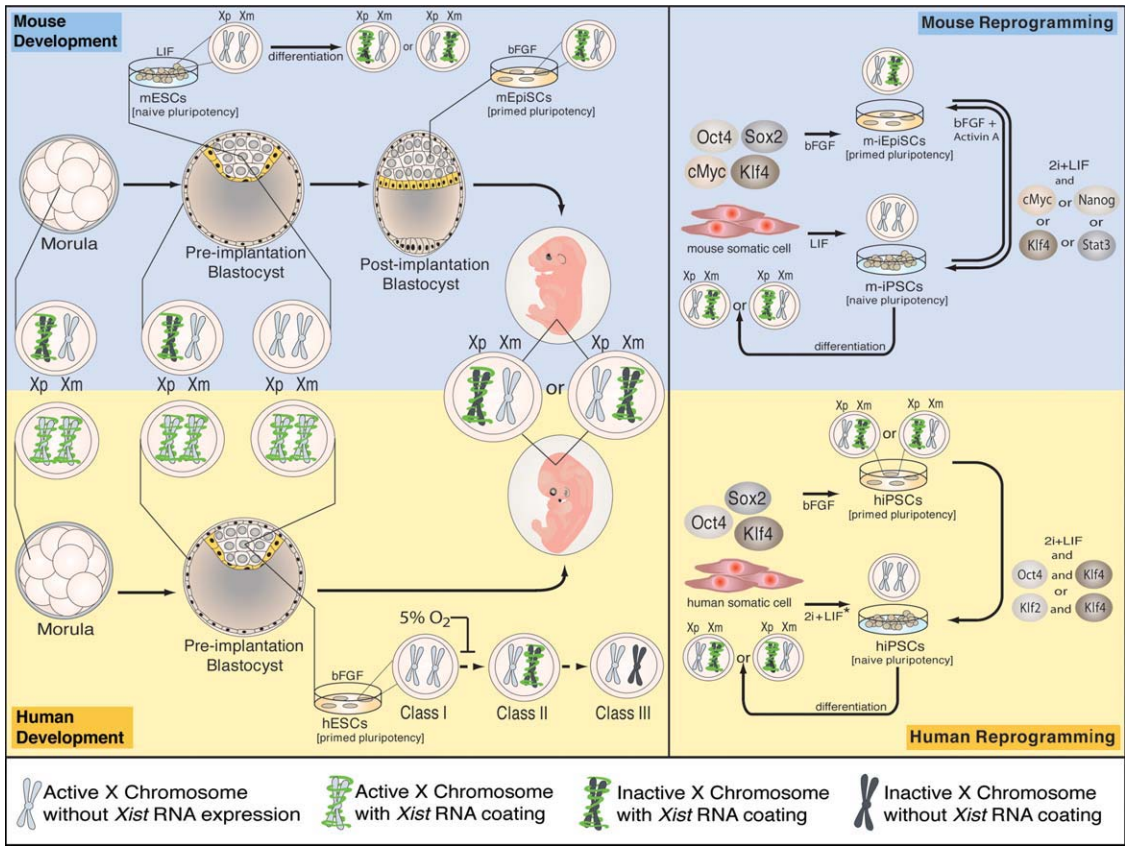

Figure 1. Mouse and human X chromosome inactivation in development and reprogramming. *The naïve human state can also be generated by overexpression of Oct4, Sox2, Nanog, and Lin28 and appears to require continuous ectopic expression of reprogramming factors for stability. Abbreviations: bFGF, basic fibroblast growth factor; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; LIF, leukemia inhibitory factor; mESCs, mouse embryonic stem cells; mEpiSCs, m-iEpiSCs, mouse epiblast stem cells; miPSCs, mouse induced pluripotent stem cells.

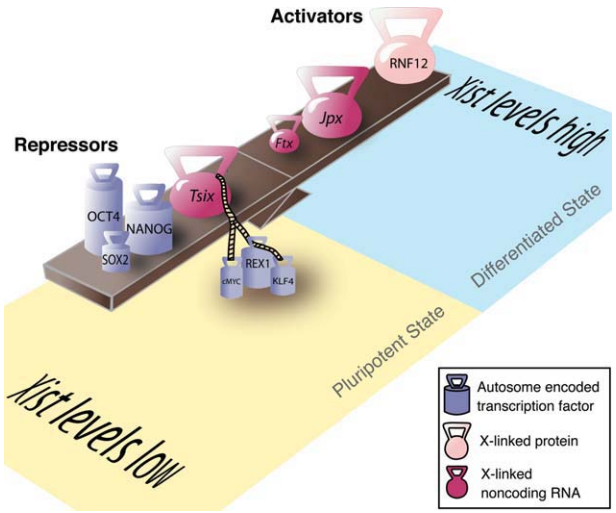

Figure 2. *Xist* activators and repressors regulate initiation of X chromosome inactivation (XCI) in mouse embryonic stem cells (mESCs). *Xist* levels are low in undifferentiated mESCs before onset of XCI, because of pluripotency transcription factors repressing *Xist* directly or indirectly via *Tsix*. X-linked *Xist* activators increase *Xist* levels during differentiation, as they themselves are upregulated. Levels of autosomal factors such as pluripotency transcription factors decrease on differentiation. Sizes and positions of weights are reflective of magnitude of *Xist* upregulation or downregulation phenotypes from experimental data (see text for discussion).

genes. An attractive hypothesis for how pluripotency is directly linked to *Xist* repression has come from a study that demonstrates binding of Oct4, Sox2, and Nanog to the first intron (intron1) of *Xist* in male and female mESCs and loss of this interaction on differentiation [35]. Intriguingly, depletion of Nanog or Oct4 leads to inappropriate *Xist* upregulation in male mESCs or biallelic *Xist* upregulation in differentiating female mESCs [35, 36]. It is still an open question whether specific binding at intron1 is at the heart of this XCI phenotype as these pluripotency transcription factors bind and regulate thousands of loci in the genome to maintain pluripotency. Mechanistically, the repressive function binding to intron1 has on *Xist* expression remains unclear, although one possibility is modification of the three-dimensional chromatin configuration within the *Xist* locus [37].

Already one study reports no effect of heterozygous deletion of intron1 and a very subtle skewing of XCI to the intron1-deleted X chromosome late in differentiation [38]. Conceivably, synergism of pluripotency factor binding to intron1 of *Xist* as well as other regulatory regions could suppress XCI in mESCs. In line with this model, *Tsix* transcription, particularly transcriptional elongation, is dependent on binding of the pluripotency transcription factors Rex1, Klf4, and cMyc, within a mini-satellite region of the regulatory region of the gene, and to a lesser extent by binding of Oct4 and Sox2, with the latter being somewhat debated [36, 39]. Thus, the pluripotency network may directly repress *Xist* and activate *Tsix*, which in turn contributes to the suppression of *Xist* and XCI (Fig. 2), an idea that could be tested with

double knockout studies of intron1 and *Tsix*. Nevertheless, it may be challenging to pinpoint the role of pluripotency regulators in XCI especially as additional *Xist* activators and repressors are discovered (see below) and transactivation or repression of these other factors by pluripotency regulators may indirectly exert XCI effects.

XCI in Differentiating Female Mouse ESCs Is Governed by a Balance of *Xist* Activators and Repressors

The mechanisms governing *Xist* upregulation during XCI must also ensure that only one X is silenced in female cells during differentiation. In addition to the X:X pairing model described above, another model proposes that in random XCI every individual X has an independent probability to initiate silencing, and this probability is proportional to the X:autosome ratio, keeping one X active per diploid chromosome set [40]. Accordingly, repressors of XCI would be autosomally encoded and activators would be X-linked. In XX cells, the double dose of the activator would stimulate *Xist* upregulation and XCI on one X, and the reciprocal *cis* silencing of the X-linked activator gene would in turn protect the other X from inactivation [40].

Rnf12, the first such characterized X-linked activator of XCI, resides $\sim$ 500 kb from *Xist* and encodes an E3 ubiquitin ligase bearing a RING domain. In line with a role in the initiation of XCI, Rnf12 protein levels increase in differentiation, and overexpression of Rnf12 stimulates ectopic XCI [41]. The heterozygous mutation of *Rnf12* in female mESCs reduces the number of female cells undergoing XCI; however, it remains unclear if there is an essential requirement for Rnf12 in random XCI as the two published homozygous knockout strategies show contrasting results of delayed differentiation and dramatic loss of XCI [38, 41, 42]. These differences may be attributed to differentiation protocols as the late appearance of *Xist* RNA cloud-positive cells suggests a selective outgrowth of cells undergoing XCI independently of Rnf12. Gene expression profiling suggests that Rnf12 acts on *Xist*, as *Xist* was the only transcript significantly downregulated in Rnf12 knockout cells [38]. Proteomic studies will likely be necessary to see if Rnf12 plays an indirect role in XCI through ubiquitylation targets.

Recently, two additional noncoding RNAs have also been identified as X-linked *Xist* activators. *Jpx*, located upstream of *Xist*, escapes XCI and increases $\sim$ 10-fold during mESC differentiation. Its heterozygous deletion leads to loss of XCI and subsequent cell death on embryoid body differentiation of female X_aX_a mESCs [43]. These phenotypes can be rescued by an autosomal *Jpx* transgene, indicating that this novel gene can function in *trans*, which contrasts *Xist* and *Tsix* [43]. Strikingly, the double knockout of *Jpx* and *Tsix* completely restores XCI kinetics and viability and will be exciting to see how this observation and the mechanistic action of *Jpx* is explained [43]. Like *Jpx*, the noncoding transcript encoded by the neighboring *Ftx* gene is also transcriptionally upregulated with female mESC differentiation. Targeted deletion of *Ftx* suggests that its role is in controlling the chromatin structure of the *Xist* promoter [44]. It is tempting to speculate that continuous expression of these noncoding transcripts may be necessary for *Xist* itself to escape XCI. *Rnf12* and *Jpx* are both bound by Oct4, Sox2, and Nanog in mESCs, suggesting that pluripotency factors could also act on XCI through these X-linked activators [45].

In summary, the activation of *Xist*, repression of *Tsix*, and XCI during mESC differentiation depends on the downregulation of pluripotency factors and the expression of X-linked activators such as Rnf12, *Jpx*, and *Ftx*, linking XCI status to the global pluripotency gene-expression network and ensuring sex-specificity of the developmental process.

XCI in Human Development

Studies on XCI in human pluripotent cells have been more limited in scope because of technical challenges in manipulating human preimplantation embryos and the ethical challenges of acquiring them. However, studies of XCI in the human system remain essential because the XCI process appears to be different from that in the mouse. For instance, human preimplantation embryos demonstrate *XIST* expression from both X chromosomes and human full-term placentas have random, rather than imprinted XCI as is found in mice [46, 47] (Fig. 1).

RNA fluorescence in situ hybridization (FISH) shows *XIST* activation as a transition from a pinpoint signal to a *XIST* RNA cloud that can be appreciated in human female preimplantation embryos as early as the eight-cell stage [48]. In one study, the majority of these *XIST* RNA-coated chromosomes show features of transcriptional silencing and enrichment of *XIST*-dependent repressive histone marks in the morula [48]. Contradictory results come from a more recent study which finds that the trophectoderm and the ICM of both female and male human preimplantation blastocysts carry active X chromosomes coated by *XIST* RNA [49]. The discrepancy between the two studies may be due to different culture conditions as well as hybridization efficiencies in the FISH procedure. Regardless, it appears that there is no imprinted XCI in human embryogenesis, that human XCI has different developmental timing, and that *XIST* RNA coating of the X and XCI are uncoupled in early human embryos (Fig. 1).

Studies of additional factors involved in human XCI are limited to *TSIX*, which may not play a functional role in human cells. *TSIX* is transcribed in fetal cells, term placenta, and human ESCs but is truncated and lacks the CpG island essential for expression in mouse cells [50, 51]. As in human preimplantation development *XIST* expression appears to be uncoupled from XCI, *TSIX*-mediated regulation may be unnecessary. However, *TSIX* has not been studied in human preimplantation blastocysts during initiation of XCI; therefore, a potential role may have been missed [52]. Other modulators of XCI in the mouse, namely *JPX*, *FTX*, and RNF12, have been mapped in the human genome but their functions have not yet been tested, mostly due to the lack of an in vitro system that allows their mechanistic dissection (see below).

Different XCI States Are Found in Human ESCs

XCI state in human ESCs (hESCs) is complicated by a gradual drift so that one hESC line can exhibit different states of XCI [53–56]. hESCs are grouped into three classes to describe the XCI states that are typically observed (Fig. 1) [53]. Class I hESCs are X_aX_a and upregulate *XIST* and undergo XCI on differentiation, similar to mESCs. This class seems to be the most difficult to stabilize in vitro because they readily switch to class II, which have initiated XCI already in the undifferentiated state and carry a *XIST*-coated X_i. Class II hESCs often further switch to class III where the silent state of the X_i is largely maintained but *XIST* is lost from the X_i along with the *XIST*-dependent histone mark

H3K27me [3], which leads to partial reactivation of some X_i-linked genes [54, 56]. *XIST* likely becomes silenced by methylation of its promoter region, and class III hESCs do not re-express *XIST* on differentiation [57, 53]. Given that both class I and III hESCs do not express *XIST* and lack an X_i enrichment of H3K27me [3], extrapolating the XCI state solely on the basis of lack of *XIST* RNA FISH or H3K27me [3] signal or even global gene expression data, has obfuscated the collective understanding of XCI in hESCs. Rather, characterization of XCI in hESC requires validation against the gold-standard assays of RNA FISH for monoallelic or biallelic expression of X-linked genes in addition to *XIST*.

hESCs derived and maintained in hypoxia, which is thought to better represent physiologic oxygen tension in development, preferentially remain in class I as demonstrated by RNA FISH for *XIST* and X-linked genes [56]. A switch to atmospheric oxygen tensions leads to irreversible transition to class II and subsequently to class III, strengthening the observation that female hESCs are unstable with respect to their XCI state (Fig. 1) [56]. It will be important to determine whether this fluctuating XCI status is indicative of global epigenetic instability in hESCs.

X Chromosome State in Human iPSCs

Like in the mouse, human iPSCs (hiPSCs) are similar to their hESC equivalent based on functional assays of pluripotency, genome-wide expression and chromatin analysis, and XCI state. At early passage, hiPSCs are class II (X_aX_i with *XIST* RNA coating) which readily switch to class III as *XIST* RNA is lost from the X_i (Fig. 1) [58]. The same X chromosome is inactivated in all cells of a given hIPSC line reflecting the origin from a single somatic cell [58, 59]. These results suggest the absence of X_i reactivation during human cell reprogramming and enable the generation of hiPSC lines expressing either only the X_m or X_p [58]. Such approaches have allowed for generation of genetically matched hiPSC lines expressing either the mutant or wild-type X-linked gene MECP2 from fibroblasts of female patients with Rett syndrome [59, 60]. However, complete skewing of XCI to one X chromosome occurs on extended passaging of fibroblasts, preventing the generation of hiPSC lines with different X chromosomes inactivated [59]. Two contradictory studies that report X_i reactivation in a subset of hiPSC lines have not performed the single cell FISH analysis of X-linked gene expression, and the skewed XCI in neurons generated from hiPSCs in one of the studies would be consistent with the lack of X_i reactivation [61, 62]. Nevertheless, these results do not exclude that different culture and reprogramming conditions could lead to XCR during hiPSC induction.

Naïve Versus Primed Pluripotency

The different XCI states in mESCs, hESCs, and iPSCs suggest that either there have been significant changes to XCI in mammalian evolution or, alternatively, that these XCI states are reflective of two different developmental states "suspended" ex vivo through current ESC culturing techniques. Although pluripotent cells by definition can give rise to cells of all three germ layers, distinct states of pluripotency have recently been described in vitro, represented by mESCs and mouse epiblast stem cells (mEpiSCs). mESCs, derived from epiblast cells of preimplantation blastocysts, are cultured in the presence of the cytokine leukemia inhibitory factor (LIF) whereas mEpiSCs are obtained from postimplantation epiblast and cultured in basic fibroblast growth factor (bFGF), in the absence of LIF. As mEpiSCs express genes associated with early events in differentiation they are considered to be in the "primed" pluripotent state, whereas the typical mESC is in the "naïve" pluripotent state [63]. mEpiSCs resemble class II hESC/iPSCs in many aspects including their flat colony morphology, bFGF culture requirement, and the presence of an X_i coated by *Xist* RNA and enriched for H3K27me [3] and the Polycomb protein Ezh2 [64–66]. X_iX_a mEpiSCs can also be generated from preimplantation blastocysts cultured with bFGF (just like hESCs), differentiated from mESCs with bFGF and Activin A, and obtained via reprogramming of fibroblasts with Oct4, Sox2, Klf4, and cMyc in bFGF-containing media as opposed to LIF [66–68] (Fig. 1B). Together, the parallels between hESCs and mEpiSCs suggest that the culture of human pluripotent cells has been optimized for the primed state and not for the naïve state.

More research is necessary to molecularly define whether mEpiSCs exhibit different types of XCI states as do hESCs/iPSCs. Interestingly, it appears that compared with mouse fibroblasts, the form of XCI in mEpiSCs is a developmental intermediate and more labile with regard to reactivation based on studies transplanting nuclei into Xenopus germinal vesicles [65]. In this reprogramming system, the X_i of female mEpiSCs is receptive to nuclear reprogramming whereas the mouse fibroblast macroH2A-enriched X_i is resistant [65].

Molecular manipulation can transition mEpiSCs to the naïve pluripotent state and these approaches have been extended to the human system to generate X_aX_a hESCs and hiPSCs. The reprogramming of mEpiSCs to an mESC-like state is achieved through a combination of ectopic expression of any one of the transcription factors Klf4, cMyc, Stat3, or Nanog and addition of LIF and 2i (a combination of two small molecules inhibiting glycogen synthase kinase 3β in the Wnt signaling pathway and mitogen-activated protein kinase signaling, which is thought to promote naïve pluripotency) (Fig. 1) [22, 66, 69-71]. A subsequent study applied this approach to hESCs and found similar requirements for acquisition of naïve pluripotency in primed hESCs when Klf4 and Klf2 or Klf4 and Oct4 are overexpressed [72]. Prolonged maintenance of the naïve human pluripotent state appears to depend on constitutive overexpression of the reprogramming factors, indicating that the naïve human state is metastable [72, 59]. As expected from the mouse system, naïve human pluripotent stem cells are X_aX_a without *XIST* expression and diverge from primed pluripotent cells in both culture requirements and molecular profile as determined by gene expression microarrays [72]. As in the mouse, *XIST* is re-expressed and random XCI initiated on differentiation of naïve human cells [59, 72]. The derivation of X_aX_a human pluripotent cells, either in the primed state under hypoxic conditions or in the naïve state, should in the future allow the modeling of initiation of XCI ex vivo.

Conclusion

However, the relevance of modeling human XCI ex vivo for the XCI process occurring during human embryonic development is still unclear. During derivation and culture of human pluripotent cells, the XCI state diverges from that described for preimplantation embryos, as the X_aX_a pattern with biallelic *XIST* coating of preimplantation embryos has not been detected in cell cultures ex vivo. Therefore, more studies are warranted but, with the approaches of these recent studies, we can already begin to define the molecular interplay of

pluripotency and XCI, akin to the mouse system, and extend these findings to optimize reprogramming to pluripotency.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflict of interests.

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