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Review

Developmental regulation of X-chromosome inactivation

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ABSTRACT

With the emergence of sex-determination by sex chromosomes, which differ in composition and number between males and females, appeared the need to equalize X-chromosomal gene dosage between the sexes. Mammals have devised the strategy of X-chromosome inactivation (XCI), in which one of the two X-chromosomes is rendered transcriptionally silent in females. In the mouse, the best-studied model organism with respect to XCI, this inactivation process occurs in different forms, imprinted and random, interspersed by periods of X-chromosome reactivation (XCR), which is needed to switch between the different modes of XCI. In this review, I describe the recent advances with respect to the developmental control of XCI and XCR and in particular their link to differentiation and pluripotency. Furthermore, I review the mechanisms, which influence the timing and choice, with which one of the two X-chromosomes is chosen for inactivation during random XCI. This has an impact on how females are mosaics with regard to which X-chromosome is active in different cells, which has implications on the severity of diseases caused by X-linked mutations.

Contents

1. Introduction

Epigenetic marks serve as memory for the ON- and OFF-state of genes and thereby maintain cellular identity across cell divisions [1,2]. The mammalian X-chromosome provides an unusual example for epigenetic regulation, as it can switch from an active to an inactive state affecting all the genes on a chromosome-wide level, with the exception of few so-called escapee genes [3]. The process of X-chromosome inactivation (XCI) thereby induces the OFF-state, which affects one of the two X-chromosomes in females, in order to ensure the same X-linked gene dosage levels as in males, which have only one X-chromosome [3–5]. XCI is a tightly controlled, multilayered epigenetic event essential for mouse development [6], and defects in XCI maintenance are associated with cancer in mice and humans [7–9]. XCI is not a permanent state, as it is reversed in the mouse embryo by X-chromosome reactivation (XCR), which occurs in the pluripotent epiblast and in the germ cell lineage [10,11]. *In vitro*, XCR is associated with mouse pluripotent stem cells like embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). When being differentiated, they undergo XCI, reflecting the events during differentiation in female postimplantation mouse development. XCI and XCR are thereby linked to

Abbreviations: E, embryonic day of development post fertilization; EpiSCs, epiblast stem cells; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; MSCI, meiotic sex chromosome inactivation; PE, primitive endoderm; PGCs, primordial germ cells; PRC2, polycomb repressive Complex 2; TE, trophectoderm; *Xic*, *X-inactivation center*; XCI, X-chromosome inactivation; XCR, X-chromosome reactivation; Xp, paternally inherited X-chromosome; Xm, maternally inherited X-chromosome; Xi, inactive X-chromosome; Xist, X-inactive specific transcript; Xa, active X-chromosome; *Xce*, *X-chromosome controlling element*.

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differentiation and pluripotency, respectively, and the molecular intertwining between X-chromosome dosage compensation and the cellular differentiation state is recently becoming increasingly uncovered. At the core of XCI/XCR regulation lies the *X-inactivation center* (*Xic*), which is a locus on the X-chromosome containing a number of mostly non-coding RNA genes, the expression of which is controlled by pluripotency factors [10]. The most prominent long non-coding RNA at the *Xic* is Xist (X-inactive specific transcript), which is expressed only from the future inactive X-chromosome (Xi), which Xist coats and silences *in cis* [12–15]. Xist initiates the XCI process by recruiting epigenetic regulators and by changing the 3D structure of the X-chromosome [16–19]. This leads to establishment of the unique epigenetic makeup of the Xi, which includes chromosome-wide enrichment for repressive marks like histone H3 lysine 27 tri-methylation (H3K27me3), histone H2A lysine 119 mono-ubiquitinilation (H2AK119ub1) histone H4 lysine 20 mono-methylation (H4K20me1), DNA methylation of X-linked gene promoters and incorporation of the histone variant macroH2A [5,20]. Combined, this ensures faithful maintenance of XCI until it is reversed by XCR again.

In this review, I will summarize, what is known about the crosstalk between pluripotency/differentiation and the XCR/XCI state during mouse development and in cell culture systems. Interesting in this aspect is the hematopoietic lineage, which displays some properties in regard to XCI, which are normally associated with pluripotent cells only [21–23]. Furthermore, I will give an overview of the causes and consequences of the mosaicism of females in respect to their X-chromosome activity. This is caused due to skewing of random XCI, where either the paternal or maternal X is inactivated. The deviation from randomness has helped to define regions on the X-chromosome, which regulate the likelihood of an X to be chosen to be either active or inactive.

2. Imprinted XCI

XCI occurs in two distinct forms during mouse development – imprinted and random. Imprinted XCI (Fig. 1A), in which always the paternally inherited X (Xp) is inactivated, takes place in the early embryo during preimplantation development, when Xist becomes expressed on the Xp from the 2-cell stage onwards [24,25] and is maintained in the extraembryonic tissues of the placenta [26]. After imprinted XCI has been erased by X-chromosome reactivation (XCR) in the epiblast of the late blastocyst (Fig. 2A) [25,27], random XCI occurs in the differentiating postimplantation epiblast (Fig. 2B) [28,29], in which either the maternal or paternal X is randomly chosen for inactivation. Imprinted XCI is essential for development, as female embryos defective in *Xist* [6] or the polycomb protein Eed [30], fail in imprinted XCI and die during postimplantation development due to placental defects. Nevertheless, imprinted XCI seems to be less strictly controlled in the placenta than random XCI in the embryo. X-linked genes can get spontaneously reactivated in trophoblast giant cells *in vivo* [31,32] and also in trophoblast stem cells *in vitro* [33], in which imprinted XCI can even be transiently completely reversed [34]. The reason for this infidelity in silencing could be the unusual chromatin state of the Xp in extraembryonic tissues, which consists both of repressive as well as active chromatin marks [32] and shows less involvement of DNA-methylation, when compared to random XCI [35].

The imprint for XCI in the preimplantation stages is set up in the germ line, but it has been a matter of debate, if exclusively in the maternal [36], in the paternal [24], or in both parental germ lines [37]. The maternal imprint (Fig. 1B) is required to ensure that *Xist* is kept silent on the maternally inherited X-chromosome (Xm), so that the Xm remains active. Evidence for this comes from parthenogenetic mouse embryos, in which both X-chromosomes are maternally inherited and are kept active during early preimplantation development until the morula stage, when the imprint seems to be overcome or erased and Xist starts to become expressed [38]. Nuclear transfer experiments have shown that the repressive maternal imprint on *Xist* is established late during oocyte development [39,40], as embryos established with non-growing oocyte nuclei expressed *Xist*, while embryos with nuclei from fully-grown oocytes did not. Mechanistically, trimethylation of histone H3 lysine 9 (H3K9me3) at the *Xist* promoter on the Xm in preimplantation embryos is critical to keep *Xist* silent and thereby the Xm active [41]. The H3K9me3 mark is required to counteract the function of the *Xist* activator RNF12/RLIM on the Xm [41], which is deposited maternally in the oocyte and which is required for expression of *Xist* on the Xp during imprinted XCI [42]. However, it seems that H3K9me3 is not the primary imprinting mark, as there is no difference in H3K9me3 at the *Xist* promoter before and after oocyte growth, when the imprint is established [43]. On the other hand, the *Xist* locus appears more condensed after imprint establishment during oocyte growth, which could influence *Xist* repression on the Xm, although the opposite has been postulated for *Xist* expression from the Xp (see below Ref. [44]). It still remains open, what the exact nature of the maternal repressive imprint on *Xist* is and how it is established.

Another critical regulator with a function in imprinted XCI is *Tsix*, the noncoding antisense regulator of *Xist*. *Tsix* is expressed from the Xm during imprinted XCI and mouse embryos with a *Tsix* mutation on the Xm express Xist from both the paternal and maternal X-chromosomes in extraembryonic tissues and die during early postimplantation development [45,46]. During preimplantation stages, *Tsix* is not required for correct Xp-specific imprinted Xist expression [47,48], therefore its function for imprinted XCI seems to be mainly restricted to the extraembryonic tissues. DNA-methylation, which is essential for autosomal imprints, is not believed to play a major role in the maternal repressive imprint on *Xist*, as maternal deletion of the *de novo* DNA methyltransferase genes *Dnmt3a* and *Dnmt3b* does not interfere with imprinted XCI [49]. Nevertheless, depletion of the DNA-methyltransferase DNMT1o, which is deposited in the oocyte and needed for maintenance of autosomal imprints during preimplantation development, is also important for imprinted XCI in extraembryonic tissues [50]. *Tsix* and its enhancer *Xite* [51], which are normally expressed only from the Xm in placentae, are expressed both from the Xp and the Xm in the absence of DNMT1o, resulting in repression of *Xist* and biallelic expression of X-linked genes [50]. The major function of DNMT1o thereby seems to be to maintain a DNA-methylation imprint on *Xite* during preimplantation development, which is established during spermatogenesis (Fig. 1C) and therefore specific to the Xp [52]. The mechanism of the establishment of the paternal imprint on *Xite* and *Tsix* still needs to be investigated.

Further indications for paternal imprinting of the Xp (Fig. 1C) come from a recent study using transgenic experiments [44]. Transgenes, containing the *Xic* region including *Xist*, *Jpx*, *Tsix* and *Xite*, have been inserted on autosomes and showed correct imprinted *Xist* expression – silent *Xist* when inherited through the maternal and active *Xist* when through the paternal germline. A similar study using single-copy transgenes has previously concluded that this is evidence that the imposed imprint is exclusively a maternal, repressive one and that *Xist* is expressed by default when inherited paternally without the need of a paternal imprint [36]. In the more recent study [44], however, in which multicopy transgenes were used, a different conclusion was drawn, which implied both a maternal and a paternal imprint (Fig. 1). When the transgene array was inherited from a father, which was homozygous for the transgene, transgenic *Xist* was not efficiently upregulated during preimplantation development in the offspring. However, when the transgene was inherited from a hemizygous father