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Xist function: bridging chromatin and stem cells

Anton Wutz

Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, 1030 Vienna, Austria

In mammals, dosage compensation is achieved by transcriptional silencing of one of the two female X chromosomes. X inactivation is dynamically regulated in development. The non-coding *Xist* RNA localizes to the inactive X, initiates gene repression in the early embryo, and later stabilizes the inactive state. Different functions of *Xist* are observed depending on which epigenetic regulatory pathways are active in a given cell. Because *Xist* has evolved recently, with the origin of placental mammals, the underlying pathways are also important in regulating developmental control genes. This review emphasizes the opportunity that *Xist* provides to functionally define epigenetic transitions in development, to understand cell identity, pluripotency and stem cell differentiation.

Epigenetic regulation of cell identity

The genome contains the information necessary to produce all cell types of an organism and for specifying their diverse cellular functions. In this manner, the body plan comprises instructions regulating cell identity and proliferation for generating the cells that make up the various organs. Therefore, for successful development of all multicellular organisms, cell proliferation and identity need to be tightly linked. Embryonic cells can show considerable plasticity, enabling them to give rise to a broad range of specialized cell types as they multiply. This developmental plasticity is reflected in the flexibility to adjust gene expression patterns, which in turn is paralleled by a more dynamic behavior of chromatin in embryonic cells [1].

Diverse adult stem cell types and stem cell niches have been described that are essential for maintenance of body function [2]; in these niches, stem cell identity needs to be faithfully maintained for the whole life of the organism [3]. Adult stem cells need to be able to change their identity to replenish mature specialized cell types contained in various organs. For instance, all blood cell lineages are constantly regenerated from a defined hematopoietic stem cell. The products of cell differentiation are specialized cell types that should no longer change their characteristic functions. Maintaining the identity of differentiated cells is important for organ function and to avoid malignancy [4].

The composition and function of chromatin are regulated during development, as shown by the dynamics of chromatin proteins and epigenetic modifications [3,5]. Depending on the differentiation state, the cell has a specific epigenetic context (see Glossary) that either enables or prevents the changing of its identity. Thus, the repertoire of mechanisms (e.g. DNA methylation) enabling epigenetic programming of gene expression patterns are predicted to constantly change as lineages differentiate. Dosage compensation systems, such as X-chromosome inactivation, show the functions of the cell nucleus in regulating gene expression in a chromosome-wide manner [6]. In mammals the 'dosage' between XY male and XX female cells is adjusted by chromosome-wide transcriptional silencing of one of the two female X chromosomes [7] (Box 1). X inactivation is under developmental control and dosage compensation is established early in the female embryo before cell fates are specified at gastrulation. An active and an inactive (Xi) X chromosome are then maintained at the same time in the female cell nucleus. The inactivation spreads over one X chromosome but not neighboring chromosomes or the other X chromosome. This indicates a mechanism that can sense the border of entire chromosomes (reviewed in Ref. [8]). Recent observations have implicated known epigenetic regulators, such as Polycomb group (PcG) genes and DNA cytosine methyltransferases, in X inactivation and taught new lessons

Glossary

Chromosome territory: the space or volume a chromosome occupies in the interphase cell nucleus. These territories have no clearly defined borders.

Epigenetic mechanisms: these regulate gene expression by making regions of the genome accessible or closing them up. Several epigenetic marks are known. These can be established directly on the DNA, as is the case for DNA cytosine methylation, or they can be established along the chromatin by post-translational modifications on histones or by binding of chromatin regulatory proteins. The structure and position of chromatin within the nucleus can also contribute to regulate gene expression. Epigenetic mechanisms include pathways to impose epigenetic information or marks, maintain them through cell division, and erase them. The activity of each of these pathways is developmentally controlled, such that a given cell has a define set of epigenetic pathways.

Epigenetic context: the sum of all epigenetic mechanisms and pathways of a given cell or cell type.

Gastrulation: a phase in early embryo development during which the morphology of the embryo is dramatically restructured by cell migration. The three germ layers, ectoderm, mesoderm and endoderm, are generated, which contain cell types that will form the definitive tissues of the embryo.

Genital ridges: the precursors of the gonads in the developing embryo. The germ cells (PGCs) migrate into the genital ridges and mature there to give rise to the gametes.

Genomic imprinting: a phenomenon involved in the control of a small proportion of genes in the genome, where the allele that is expressed is determined solely by which parent contributes it. Imprinted expression is monoallelic from a single parentally inherited allele of a gene, either the maternally or the paternally inherited allele.

Primordial germ cells (PGCs): the precursors that will form the gametes. PGCs are specified outside the gastrulating embryo and then migrate into the genital ridges, where they mature and form the germline cells.

Corresponding author: Wutz, A. (wutz@imp.univie.ac.at).
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Box 1. X inactivation in human and mouse

Random X inactivation is thought to have emerged with the evolution of placental mammals [12,26]. In mice imprinted X inactivation of the paternally inherited X chromosome is found in extraembryonic lineages, whereas in humans X inactivation is believed to be random [70]. Sequences within the *Xic* are not perfectly conserved, and sequence variation is observed between *Xist* genes of different mammals. Mouse *Xist* can be observed attached on condensed chromosomes at the onset of mitosis, whereas human *Xist* is displaced before chromosome condensation [70]. In addition, elements within the *Xic* that regulate X chromosome counting and choice, such as the non-coding *Tsix* RNA, are not conserved between mouse and human [71]. In female human ES cell lines X inactivation can be initiated before differentiation [72,73]. In summary, these observations show an overall conservation of random X inactivation among different mammals with variations in some aspects of the mechanism.

about their function. Here, I discuss our current understanding of X inactivation in mice and show how this process is beginning to shed light on aspects of pluripotency and the maintenance of cell identity.

The dynamic activity state of the Xi during development

The chromatin of the Xi shows dynamic behavior as the chromosome undergoes several rounds of inactivation and reactivation in cells of the early embryo and germline (Figure 1). Initially the paternally inherited X chromosome becomes inactive during the first cleavage stages of early female mouse embryos [9–11]. Imprinted inactivation of the paternally inherited X is maintained in the extraembryonic tissues, which include the trophectoderm and primitive endoderm [12]. The inactive X is reactivated specifically in cells of the inner cell mass (ICM) of the blastocyst, which give rise to the embryo [10,13]. The developing female epiblast subsequently consists of cells with two active X chromosomes and, thus, dosage compensation is not sustained in the embryo between embryonic day 3.5 and 5.5. Before gastrulation, dosage compensation is finally established by the random inactivation of either the maternal or paternal X chromosome in all cells of the embryo [12]. Similar to the cells of the epiblast, female mouse embryonic stem (ES) cells have two active X chromosomes. These cells can reactivate a somatic Xi introduced by cell fusion, demonstrating that the remarkable reprogramming activity of the early embryo is maintained in ES cells [14]. Furthermore, upon differentiation of ES cells, random X inactivation is recapitulated. Reactivation of the Xi in ES cells and the ICM thus correlates with the establishment of pluripotency.

Once established in the embryo, the inactive state of the Xi is maintained throughout subsequent cell divisions. The Xi is remarkably stable in somatic cells of the embryo and adult [12], whereas reactivation of the Xi is observed only in the germline. Primordial germ cells (PGCs) have two active X chromosomes as they enter the genital ridge between day 11.5 and 13.5 of female embryonic development [15]. Reactivation of the Xi correlates with the reprogramming of imprints, which occurs before the imposition of new maternal imprints on the genome of the gamete (reviewed in Ref. [16]). Changes in the activity and composition of the Xi chromatin thus highlight a special cellular context with epigenetic reprogramming potential.

Xist: a trigger of chromosome wide silencing in *cis*

A single locus on chromosome X controls the inactivation of the chromosome: the X inactivation center (*Xic*). Non-coding RNAs and regulatory elements within the *Xic* have been identified that help 'count' the number of X

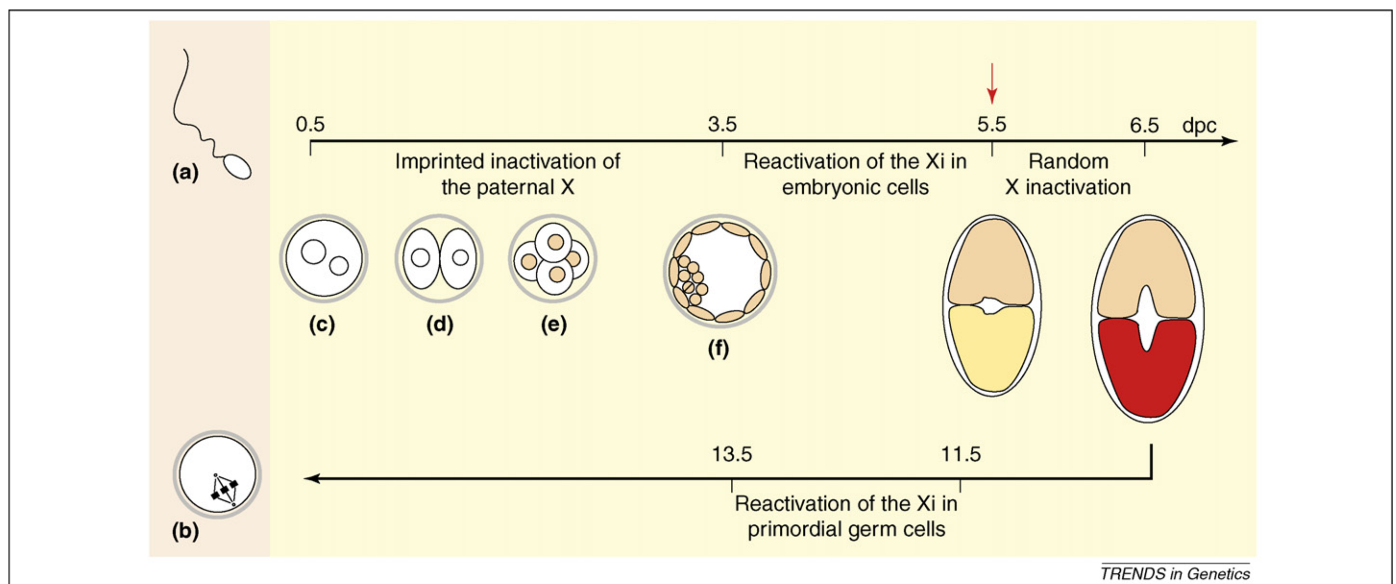

Figure 1. Developmental regulation of X inactivation. Relevant embryonic stages and the embryonic time (days post coitum, dpc) are indicated. **(a)** Male and **(b)** female gametes contribute the paternal and maternal chromosome set to the zygote **(c)** at fertilization. **(d)** A two-cell embryo, **(e)** a four- cell embryo, and **(f)** a blastocyst are shown. Inactivation of the paternally inherited X chromosome is first observed in the early cleavage stages of female mouse embryos (orange cells and tissues). Imprinted X inactivation is maintained in the extraembryonic lineages of the conceptus. In the blastocyst, cells that give rise to the embryonic lineages reactivate the Xi and the developing female epiblast consists of cells with two active X chromosomes (yellow). At gastrulation random X inactivation of either the paternal of maternal X establishes dosage compensation (red). Once established, the Xi is stably inherited throughout subsequent cell divisions in the somatic lineages. In the migrating germ cells, another round of Xi reactivation takes place and female primordial germ cells have two active X chromosomes when they reach the genital ridges. Thus, the X chromosome can undergo two rounds of inactivation and reactivation before it is passed on to the next generation.

chromosomes and choose which of the two X chromosomes should be inactivated [17]. Random X inactivation involves an elaborate mechanism for ensuring that one of the X chromosomes remains active and the other is inactivated. During this process the *Xic* regions of the two X chromosomes physically interact with each other and *Xic–Xic* pairing correlates with the initiation of X inactivation [18,19].

Chromosome-wide silencing of the X is then initiated by *Xist*, an RNA that is transcribed specifically from the *Xic* on the Xi, and 'spreads' over chromatin encompassing the territory of the Xi in *cis* [12]. In human cells, *XIST* localizes to the center of the Xi, which consists predominantly of non-genic sequences and repetitive elements [20]. Genes on the active and inactive X chromosome are located at the outer margin of the chromosome 'territory' – the space a chromosome occupies in the interphase cell nucleus. During the initiation of X inactivation in differentiating mouse ES cells, once a gene is silenced it moves to a more internal position on the Xi chromosome territory [21].

Xist RNA can 'spread' over autosomes from which it is expressed transgenically [12]. Thus, X chromosomal sequences are not strictly required for *Xist* localization. This is consistent with the apparent absence of specific DNA sequence motifs that correlate with silent sequences on the Xi [22]. However, the X chromosome contains a greater than average number of genomic repeats of the long interspersed element (LINE) class that might contribute to the spread of silencing [23,24]. Studies of X:autosome translocation chromosomes have shown that spreading of *Xist* RNA on autosomal chromatin is less efficient. In one case spreading is attenuated at the translocation breakpoint, where a low density of LINEs is observed at the autosomal partner [25]. The chromosomal context therefore has a modulatory role for the spreading of silencing, and the X chromosome might have been optimized for dosage compensation during evolution [12,26].

The specific localization pattern of *Xist* is unique among the known RNAs. Moreover, *Xist* is found only in placental mammals, which undergo random X inactivation. The sequenced genomes of marsupials or more distant vertebrates do not encode an *Xist* gene [27]. *Xist* seems to combine several functions bridging chromatin and chromosome territories [28] (Figure 2). Chromosomal association and spreading of *Xist* is mediated by functionally redundant sequences that are dispersed throughout the length of the RNA [26]. These sequences seem to act cooperatively but show little homology. This has thus far prevented the identification of specific sequence motifs that mediate the spreading of *Xist* over a chromosome. Gene silencing crucially depends on the presence of a conserved and well defined repeat sequence on the 5′ end of *Xist* [29], known as repeat A. Importantly, deletion of repeat A results in a mutant *Xist* RNA that can associate with and spread over the X chromosome but does not cause gene repression. *Xist* RNA localization can therefore be separated from silencing by specific mutations, and RNA localization is required but not sufficient for X inactivation.

Establishment of chromosome-wide silencing depends on the cellular context

Using an inducible ES cell system (Box 2) it can be observed that the silent state is initially reversible and that the chromosome will reactivate when *Xist* expression is turned off [30]. This parallels the reactivation of the paternally inherited Xi in the ICM and the ability of ES cells to reactivate a somatic Xi introduced by cell fusion. Reversibility of silencing is lost upon cellular differentiation and *Xist* becomes dispensable for gene repression on the Xi. Stabilization of the Xi possibly parallels changes in the dynamics of chromatin proteins observed in differentiating ES cells [5]. In somatic cells, gene repression on the Xi is maintained by several epigenetic mechanisms. It has been shown that DNA methylation, histone H4 hypoacetylation

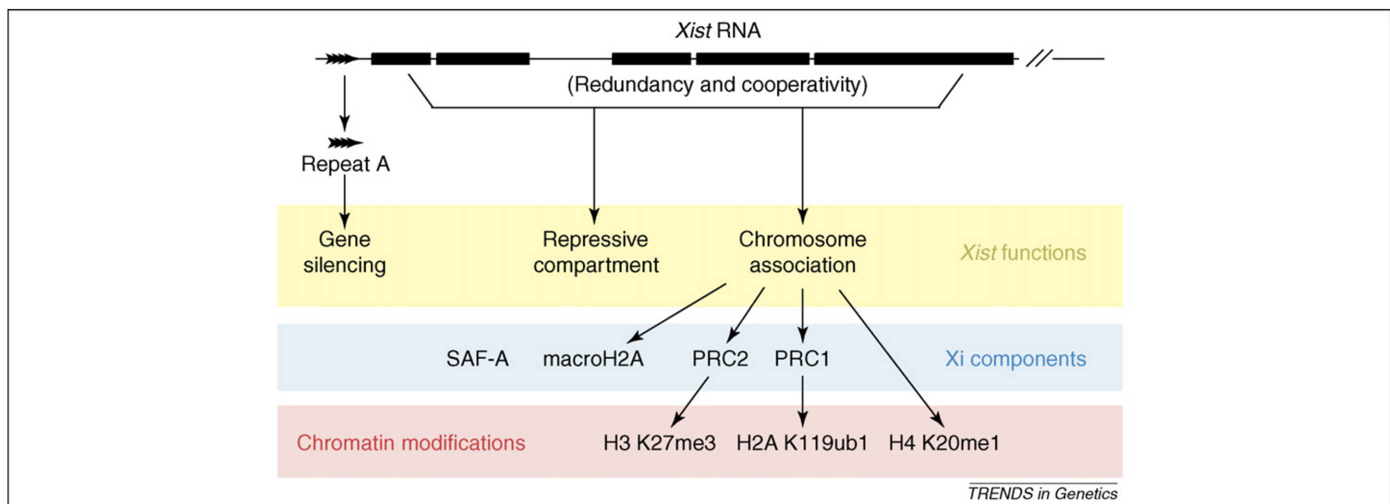

Figure 2. Functions of *Xist* RNA. Localization and spreading over the chromosome depends on multiple sequences throughout *Xist*. These sequences act in a redundant and cooperative manner, but no specific motifs have been identified for chromatin attachment or spreading. Gene repression depends on the 5′ end that contains *Xist* repeat A. The silencing function of *Xist* is not required for localization or recruitment of proteins and chromatin modifications to the Xi. A mutated *Xist*, which lacks repeat A, can still form a repressive compartment in ES cells, but genes are not silenced and do not relocate into this domain. Arrows indicate the present view of the molecular pathways that establish Xi chromatin. *Xist* accumulation leads to the formation of a repressive compartment. Recruitment of PRC2 and PRC1 by *Xist* establishes the chromosome-wide histone marks H3 K27me3 and H2A K119ub1, respectively. Furthermore, H4 K20me1 and the histone variant macroH2A are enriched on the chromosome when *Xist* is expressed.

Box 2. An inducible cell system for studying X inactivation

Xist expression can be experimentally manipulated by using a heterologous expression system in mouse ES cells (Figure Ia). A tetracycline dependent transactivator protein (nls-rtTA, blue ovals in Figure Ia) is therefore expressed from a ubiquitously active locus (R26). DNA binding of this transactivator is induced by the addition of the tetracycline analog doxycycline (dox, green squares) and triggers expression of *Xist* under control of the inducible promoter. *Xist* expression can be induced by addition of doxycycline at various time points in differentiating ES cell cultures. An induction time course is shown in Figure Ib, with expression of *Xist* early in differentiation and reinduction at a later time point. This type of experiment has proven useful for characterizing the imposition of heritable chromatin states by *Xist* in ES cell differentiation.

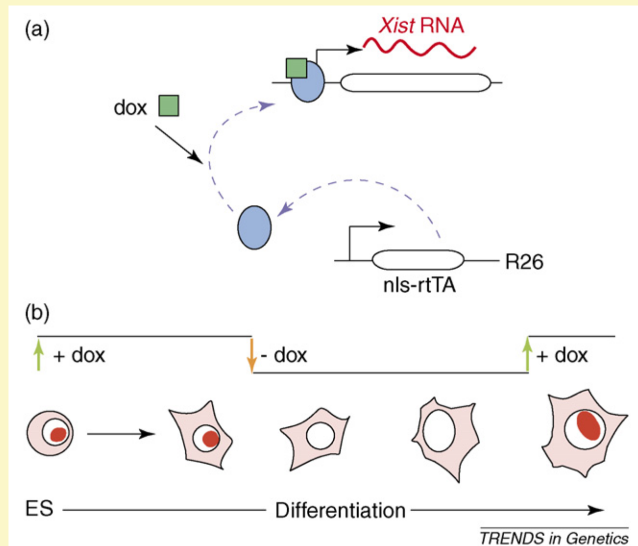

Figure I.

and chromosomal late replication contribute synergistically to the stability of the Xi [31].

In contrast to the cells of the early embryo, *Xist* does not have a silencing function in differentiated cells. When *Xist* is ectopically expressed in somatic cells that have a mutation in *Dnmt1* gene, which encodes a DNA methyltransferase, *Xist* localizes to the X chromosome but does not cause gene repression [32]. In mouse ES cells *Xist* loses the ability to initiate silencing soon after the onset of differentiation [30]. Diminishing silencing function is observed before the inactive state becomes stable and *Xist*-independent. The coordinated loss of the silencing function and the stabilization of repression indicate a change in the activity of the epigenetic regulation pathways triggered during stem cell differentiation. The silencing function of *Xist* thus provides an opportunity to examine the epigenetic context of different cell types during development.

A detailed analysis of the cellular context for the initiation of silencing has been obtained for the blood system [33] (Figure 3). Ectopic *Xist* activation from the single X chromosome in adult male mice carrying an inducible *Xist* allele results in a general hematopoietic failure. Initiation of silencing is observed in immature hematopoietic precursors, but not in the hematopoietic stem cells or mature blood cells. Therefore, the context for silencing appears transiently in hematopoietic differentiation after the onset of stem cell differentiation and lineage specification. The change in the ability of *Xist* to silence is paralleled by changes in chromatin modifications. In female mice alterations of the Xi chromatin occur in hematopoietic progenitors [33]. However, gene repression on the Xi and dosage compensation is maintained throughout hematopoietic differentiation. Therefore, the silencing function of *Xist* in hematopoietic precursors does not depend on the reprogramming ability of cells for reactivation of the Xi. This suggests that these pathways are regulated independently.

Chromosome inactivation has also been observed following ectopic *XIST* expression in a human tumor cell line [34]. Tumors have been reported to display stem cell like chromatin patterns and might differ in their epigenetic mechanisms from normal somatic cells [4]. Although silencing of tumor suppressor genes is a common mechanism that might point towards activity of epigenetic pathways (reviewed in Ref. [35]), the correlation between *Xist* silencing function and malignancy remains to be shown.

What is the epigenetic context and how does it translate to a molecular mechanism for gene silencing?

Several mammalian repression mechanisms with a potential role in X inactivation have been analyzed for their contribution to gene silencing (Figure 2). Among these are DNA methylation and Polycomb group (PcG) genes, which contribute to X inactivation in embryonic and extraembryonic lineages to different extents [36–38]. The Xi shows a heterogeneous pattern of DNA methylation, with promoter sequences being highly methylated and non-promoter sequences showing less methylation on the Xi than on the active X chromosome [39]. DNA methylation contributes to, but is not essential for, the maintenance of gene repression on the Xi [31]. Notably, disruption of *Dnmt1* in mice causes a global loss of DNA methylation in the genome and leads to reactivation of genes on the Xi during development [36]. However, DNA methylation is not required for the initiation of silencing [40].

The histone variant macroH2A has been observed in chromatin of transcriptionally inactive regions. MacroH2A is concentrated on the Xi in somatic and embryonic cells [41]. In differentiating ES cells recruitment of macroH2A occurs after silencing has been established. MacroH2A is unlikely to have a crucial role in silencing because in somatic cells its concentration depends on *Xist*, whereas silencing is independent of *Xist* [31]; in addition, mice with a mutated *macroH2A1* gene have no obvious defects in X inactivation [42].

The nuclear scaffold attachment factor A [SAF-A; also known as heterogeneous nuclear ribonucleoprotein U (hnRNP-U)] is concentrated on the Xi in human cell lines [43,44]. SAF-A contains an RNA binding domain, which is required for its recruitment to the Xi. SAF-A forms part of a stable proteinaceous structure that might help stabilize X inactivation. In mouse embryogenesis, a reduction in Saf-A protein levels owing to a hypomorphic mutation causes lethality [45], making analysis of its function in X inactivation challenging – thus, its role in silencing remains unclear.

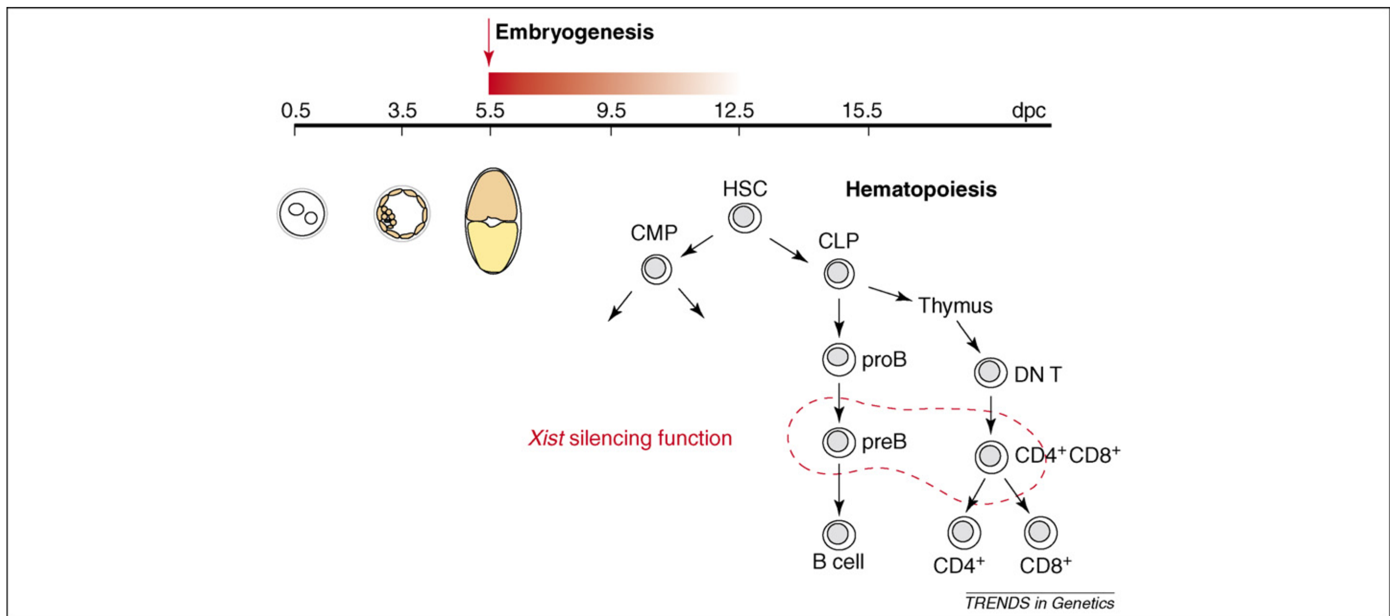

Figure 3. Developmental regulation of the gene silencing function of *Xist*. The cellular context regulates the function of *Xist* to initiate chromosome-wide gene repression. In embryogenesis random X inactivation is established at day 5.5 (red arrow). *Xist* silencing function (red) can be observed in cells up to day 12.5, with the number of cells decreasing gradually and possibly cell type specific. In most somatic cells *Xist* does not function in gene repression. In the hematopoietic stem cell (HSC) and common lymphoid (CLP) and myeloid (CMP) progenitors and more mature blood cells, *Xist* cannot initiate silencing. The silencing function of *Xist* is reestablished in committed precursors of the hematopoietic system, such as pre-B cells and T cells, which express the CD4 and CD8 surface markers (dashed red line). Thus, in the blood system the context for silencing is transiently established after onset of HSC differentiation and lineage commitment.

Recruitment of PcG proteins to the Xi is an early event in X inactivation. Polycomb repressive complex 2 (PRC2) contains the PcG proteins Eed, Ezh2 and Suz12. PRC2 causes chromosome-wide histone H3 lysine 27 tri-methylation (H3 K27me3) on the Xi [46]. A second catalytically active PcG complex containing the Ring1A and Ring1B proteins (PRC1) mediates mono-ubiquitination of histone H2A lysine 119 (H2A K119ub1) along the Xi [47,48]. H3 K27me3 and H2A K119ub1 can both be recruited by *Xist* independent of gene silencing and are thus not sufficient for gene repression [49–51]. A mutation in *Eed* causes reactivation of genes on the Xi in extraembryonic tissues in differentiating trophoblast cells [52]. However, *Eed* and PRC2 function are dispensable for X inactivation in other extraembryonic lineages and in embryonic cells [51,53]. Ring1B and H2A K119ub1 can be recruited in cells lacking *Eed*; this might explain the lack of an obvious defect in X inactivation in ES cells that have a mutated *Eed* gene [51]. Disruption of the *Ring1B* gene in mice causes an early embryonic lethality, which has precluded the analysis of its precise function in X inactivation [54,55].

None of the chromatin components or modifications studied to date seems to be essential for silencing. This indicates potential redundancy among them or suggests the existence of as yet unidentified pathways. The earliest event in X inactivation is an exclusion of the transcription machinery from the Xi chromosome territory. RNA polymerase II, splicing factors and nascent transcripts are virtually absent from the *Xist*-covered domain before histone modifications can be observed on Xi chromatin [21]. Although the formation of this repressive domain (i.e. the *Xist*-covered domain) is not sufficient for gene repression, it might be the basis of gene silencing. Genes relocate into the repressive domain upon silencing [21]. However, it is unclear whether the relocation of these genes is a cause or a consequence of silencing [56]. These observations point to entirely new epigenetic mechanisms for gene repression on the Xi. Thus, gene silencing by *Xist* might be molecularly separated from the known chromatin components and marks of the Xi.

Recruitment of Polycomb group proteins and heritable chromatin states

A hallmark of epigenetic systems is the generation of heritable chromatin states. In differentiated ES cells, *Xist* might have a role in the maintenance of chromosome-wide gene repression [31] but cannot initiate silencing. *Xist* expression is required for the recruitment of PcG proteins and macroH2A to the Xi [50,51,57]. *Xist* expression is not sufficient for the establishment of the PcG-associated chromatin modifications H3 K27me3 and H2A K119ub1. Although these modifications are efficiently established over the Xi, ectopic *Xist* expression from an active chromosome in differentiated cells leads to inefficient PcG recruitment and histone modifications. Thus, in differentiated cells chromatin influences the function of *Xist* to recruit PcG complexes.

Gene repression is not essential for the recruitment of PcG proteins and their modifications. Truncated *Xist* lacking repeat A can recruit PcG proteins and modifications at early time points in ES cell differentiation [49–51]. If *Xist* is induced after an ES cell has differentiated, its PcG recruitment function is lost. However, if *Xist* is expressed until differentiation has progressed to a certain stage, a change on the chromosome is triggered that enables efficient recruitment of PcG proteins in differentiated cells in the absence of gene silencing. Once this change in the property of the chromosome has occurred, it is fixed and maintained independent of *Xist*, thus providing a heritable epigenetic memory. The formation of this epigenetic memory

coincides with *Xist*-independent X inactivation in differentiating ES cells [50]. Although memory is not linked to gene silencing, it might be the basis for maintenance of repression on the Xi. This suggests a mechanism for maintaining a certain expression–repression profile that is established before differentiation, highlighting the establishment of heritable chromatin properties independently of gene silencing that could act as platform for gene regulation.

Epigenetic memory is maintained independently of all known components of the Xi and independent of gene repression. This indicates that as-yet unidentified and potentially novel mechanisms are involved in memory. For example, the PcG components of the Xi might have a role in memory imposition and could provide a regulatory function for changing chromosome states. The molecular mechanism for PcG recruitment seems to differ between ES cells and differentiated cells. RNA and H3 K27me3 have been described as mediating the binding of Polycomb [58]. Furthermore, PcG function is regulated by cellular signaling pathways that are linked to gene regulation and plasticity [59,60]. Conversely, PcG proteins have been implicated in regulation of pluripotency [61]. Mapping of chromatin binding sites has shown that PcG proteins are bound to promoters of developmental control genes in ES cells [58,62,63]. Observations of PcG action in X inactivation are thus relevant to understanding their function in stem cells.

Concluding remarks and future outlook

Three functions of *Xist* – localization to the chromosome, silencing and memory recruitment – can be mechanistically separated. All are required for establishing a repressed Xi and thus for dosage compensation in mammals. However, manipulating the *Xist* sequence or the cellular context can induce partial chromosomal phenotypes. For instance, a chromosomal memory in the absence of gene silencing can be induced. An active chromosome decorated with chromatin marks (e.g. H3 K27me3 and H2A K119ub1 and PcG and macroH2A proteins), which are normally found on the Xi, can be established. Conversely, an Xi can be maintained without these precise epigenetic marks. Also, localization of *Xist* RNA to the chromosome without chromatin decoration or silencing can be achieved. This shows *Xist* as a 'magic wand' that can paint chromosomes in a variety of 'colors'. Importantly, it has become clear that the mechanisms used by *Xist* are strictly dependent on specific cellular contexts (Box 3).

X inactivation can thus enable visualization of chromatin states or epigenetic 'programming' during development. Conversely, Xi reactivation can be viewed as a process of 'reprogramming', in which existing epigenetic patterns become erased in a chromosome-wide manner. It is tempting to speculate that pluripotency is achieved by a combination of pathways that control the self-renewal, programming and reprogramming of stem cells. A molecular program for ES cell pluripotency has recently been identified that involves

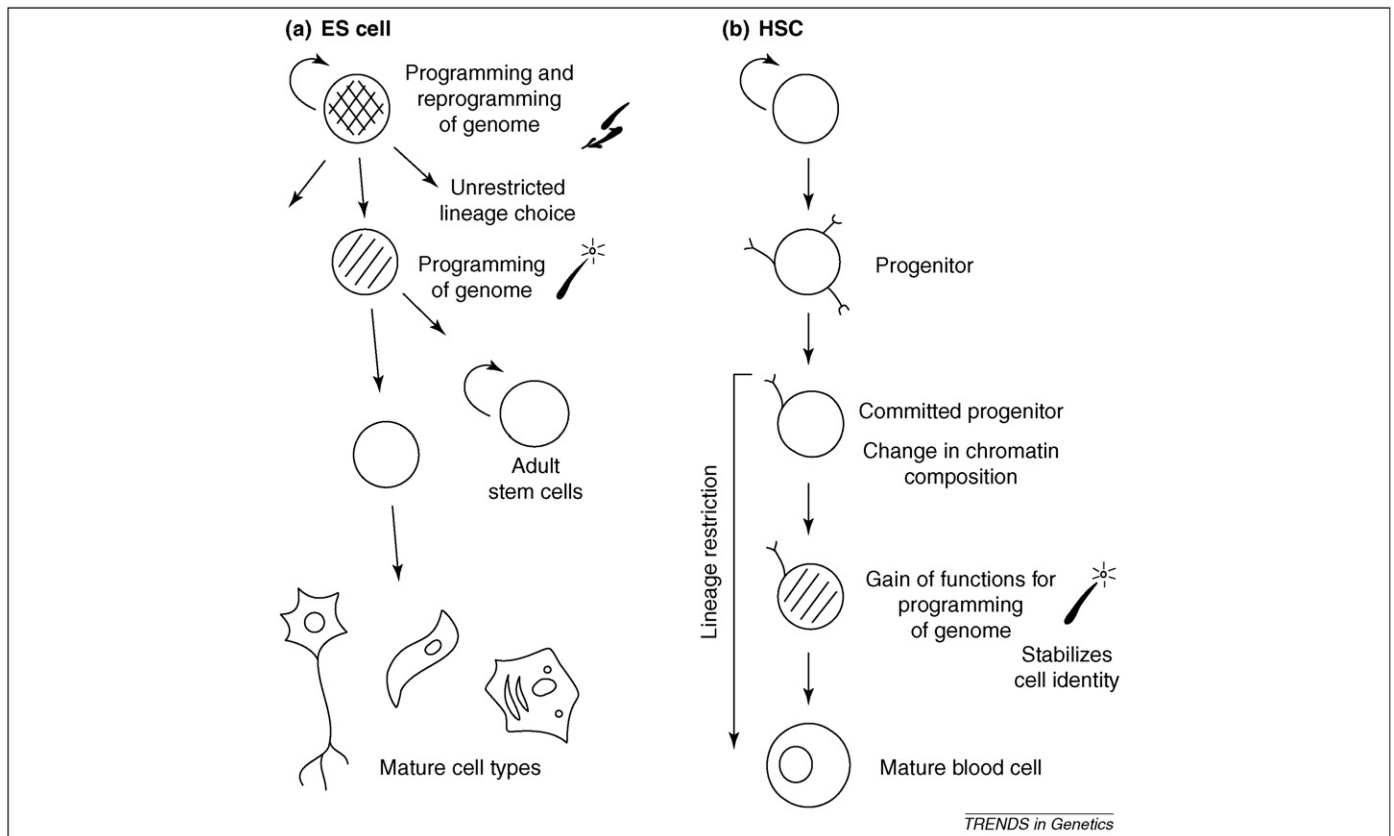

Figure 4. Epigenetic mechanisms in embryonic versus adult stem cell differentiation. A hypothetic scheme of stem cell differentiation is depicted for **(a)** embryonic stem (ES) cells and **(b)** HSCs. ES cells have a cellular context featuring *Xist* silencing function and the ability to reactivate an Xi. HSCs have none of these, but upon differentiation *Xist* silencing function is transiently established in blood precursor cells. Points at which epigenetic programming and reprogramming events might contribute to control the differentiation path by regulating the stability or plasticity of the cell type are indicated. The stages of differentiation when pathways for programming and reprogramming are activated are also indicated (hatched and cross hatched cell types).

Box 3. Important questions for future research

X inactivation has been under intense study for many years, and progress in identifying the molecular players in X inactivation has been made. Yet the pathway for gene silencing by *Xist* remains unclear and needs to be defined. Efforts thus far have yielded several proteins and epigenetic modifications that contribute to the chromatin of the Xi. However, no proteins that are specifically required for silencing have been reported.

A related question is what restricts the function of *Xist* for initiation of silencing to specific cell types. It is of importance to explore whether cells that have activated the pathways that enable *Xist* silencing function are contributing to adult stem cell niches other than those of the hematopoietic system. Furthermore, the possibility that certain types of cancer cells might share such an epigenetic context is not too remote and might provide a new avenue for therapeutic strategies in oncology.

At present there is no model that can explain how *Xist* spreading is confined to the one chromosome from which it is expressed. It needs to be clarified how spreading of *Xist* to other chromosomes is avoided. For this it seems useful to identify potential attachment sites on chromosomes for *Xist*. One would expect a mechanism that regulates *Xist* attachment on chromatin to enable spreading of the RNA over the chromosome. Progress in understanding the principle behind the specific localization pattern of *Xist* promises to shed light on how chromosome territories can be confined with the mammalian nucleus.

several stem cell transcription factors, including Oct4 and Nanog [61,64]. A considerable differentiation potential has been achieved by ectopic expression of several stem cell factors in fibroblasts, although pluripotency of the converted cells remains to be shown [65]. Recent evidence suggests that the stem cell program can be activated epigenetically by exposure of somatic cells to ES cell extracts [66]. This observation indicates a mechanistic link between the context for epigenetic reprogramming and pluripotency. One might think of it as opening up of the genome, making genes potentially expressible.

Epigenetic programming is, then, important to restrict the developmental plasticity and is crucial for the faithful maintenance of cell identity. Therefore, it has a role in stem cell differentiation. The silencing function of *Xist* in the hematopoietic system highlights the programming events that stabilize the cell identity of the maturing blood cells. The link between epigenetic programming and reprogramming is most clearly observed in the dynamic repressed state of the Xi during development, and it enables the identification of both the cellular states and the conditions under which these changes occur in mammalian development. Although embryonic cells have a reprogramming and programming potential, adult stem cells generate a programming potential on differentiation (Figure 4). There is emerging evidence that such transitions might have a role in malignancy. X inactivation has been observed in human testicular tumors [67]. Conversely, loss of the Xi is a common feature of *BRCA1* mutant and sporadic basal-like breast cancer [68]. However, it remains to be seen whether pathways for programming and reprogramming are activated during tumorigenesis [69].

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