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Review

X inactivation and disease

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ABSTRACT

X inactivation is the mechanism by which mammals adjust the X-linked gene dosage between the sexes. The dosage difference between XX females and XY males is functionally equalized by silencing one of the two X chromosomes in female cells. This dosage-compensation mechanism is based on the long functional *Xist* RNA. Here, we review our understanding of dosage compensation and *Xist* function in the context of disease.

Contents

1. Introduction

In mammals, transcriptionally silencing of one of the two X chromosomes is necessary to achieve dosage compensation. As a consequence counting the number of X chromosomes and choosing the X chromosome for inactivation is a major relevant step in this process [1].

The 17 kb non-coding RNA *Xist* triggers X inactivation. Gene silencing by *Xist* is only achieved in certain developmental contexts that can be found in cells of the early embryo and specific hematopoietic progenitors [2]. The absence of critical silencing factors may give an explanation of why *Xist* cannot silence outside these contexts.

Recent findings in X inactivation have shown that some aspects of dosage compensation are relevant for disease. In this review we will summarize aspects of pathology related to the counting and choice process of X inactivation and also the implications that the aging process might have on this phenomena. It has been recently shown that *Xist* can initiate gene silencing in lymphoma cells providing a link between X inactivation and cancer. By using this tumor context the special AT rich binding protein SATB1 has been identified as an essential silencing factor [3]. Because understanding the pathway enabling chromosomal silencing by

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Xist in cancer progenitors due to the expression of SATB1 may be relevant for cancer biology we will explore the implications of this epigenetic route. A related question is: Is the X inactivation mechanism affected during aging? By using cells from premature aging disease patients it has been observed that maintenance of X inactivation might be affected during this physiological process [4]. In this review we will describe the mechanistic steps of X inactivation and explain the current understanding of their relevance for disease.

2. Counting and choice

X chromosome inactivation (XCI) is the process by which one of the two X chromosomes becomes transcriptionally inactive in each somatic cell of mammalian females. This is the dosage-compensation mechanism to functionally equalize the imbalance of X-linked genes between XX females and XY males [5]. In mice XCI is random in the embryo, and imprinted in extra-embryonic tissues, where the paternally inherited X chromosome is inactivated [6]. The mammalian dosage-compensation system is mainly regulated by the X inactivation center (*Xic*) located on the X chromosome. The *Xic* regulates counting of the number of X chromosomes and contains the non-coding *Xist* RNA gene, which localizes to the inactive X chromosome (Xi) and triggers chromosome-wide gene repression. During the early stages of XCI, *Xist* expression is regulated by the *Tsix* gene that is a long untranslated RNA, which acts mainly in the nucleus and is transcribed in antisense direction over the *Xist* gene.

This locus may have evolved only in placental mammals [7–9]. In marsupials and the more distant vertebrates, the structure of the *Xic* genome region is different. The *Xist* gene may have emerged from an ancestral protein-coding gene *Lnx3* during mammalian evolution [10], demonstrating that XCI in marsupials needs an alternative mechanism for dosage compensation. Interestingly, no evidence of efficient dosage compensation has been found in chicken and finches [11]. There may be partial dosage compensation, just as in mammals such as monotremes.

The potential risk of female lethality dictates a need for a precise counting process. It is supposed that counting and choice in XCI can be explained by the action of an autosomal blocking factor (BF), which protects one X chromosome per diploid genome from inactivation (Fig. 1). This factor is thought to block the inactivation of one of the two X chromosomes by interaction with the *Xic*. A candidate cis acting element for this factor is *DXPas34*, which is located 3′ of *Xist* [12]. Deletion of this element results in some degree of XCI in male cells though not as efficiently as in female XX cells, indicating a role for this element in the counting process.

Although more work is needed to identify factors involved in the counting process XCI starts with the accumulation of *Xist* along the X chromosome that will become inactive. Based on the blocking factor model a second model (symmetry breaking model) has been proposed in which diffusible molecules are quantitatively sequestered on the inactive X chromosome by a mechanism that is based on intermolecular BF interactions with binding equilibration occurring fast enough to discriminate between the active blocked and the inactive not blocked X chromosome [13]. A third model (mutual exclusive choice model) takes advantage of position coordinated transregulation of interacting *Xics* as trans-interaction of X chromosomes by *Xic–Xic* pairing has been observed [14–17]. A fourth model is based on alternate epigenetic states that could mark the X chromosome, Xa or Xi, before X inactivation and could represent blocking factor binding to one chromosome or accessibility and transcriptional differences between the two *Xics* [18]. The last model is a stochastic model and it assumes that each chromosome undergoes inactivation with a certain probability. In fact chaotic choice has been observed if *Tsix* is disrupted in mice from both *Xic* alleles and females are viable with a low frequency, thus, this mechanism may be an important part for the XCI counting and choice process [19].

It is possible that both the stochastic model and the symmetry breaking models occur and perhaps the symmetry breaking model is important to ensure that that both Xs do not start to become inactivated at the same time.

3. Counting and choice: why are they relevant for disease?

The choice of which of the two X chromosomes will be inactivated is totally random and once it is initiated is propagated to the daughter cells. This phenomenon has consequences for the outcome of diseases caused by mutations of X-linked genes or by numerical or structural abnormalities of the X chromosome. Heterozygous females are a mosaic of two populations of cells expressing either the wild-type or the disease allele. Of course the expected ratio of cells expressing the mutated and the wild-type allele should be 50:50 but skewing of the XCI pattern can occur altering this ratio. Skewing can be due to positive or negative cell selection and can modulate the severity of the phenotypes in women who are carriers of X-linked mutations [20]. For example, X-linked dominant male lethal disorders are useful to explain the variability of expression of the disease phenotypes. An X-linked disorder is dominant when it is phenotypically expressed in heterozygotes and a reduced subset of them is characterized by male lethality or reduced viability in males. These disorders and the corresponding locus for these diseases are chondroplasia punctata 2, *CDPX2*; congenital hemidysplasia with ichtyosiform erythroderma and limb defects, *CHILD*; oculo-facio-cardio-dental, *OFCD*; terminal osseous dysplasia, and pigmentary defects, *ODPD*; Rett syndrome, *RTT*; incontinentia pigmenti, *IP*; oral-facial-digital type 1, *OFD1*; mycrophtalmia with linear skin defects, *MLS*; Aicrdi syndrome, *AIC* and Goltz syndrome, *FHD*; respectively [21].

Among these disorders the causal genes were identified in six cases, where two of these genes escape X inactivation in humans and four are inactivated [21]. Murine models are available for some of these diseases. In some cases the disease phenotype is related to

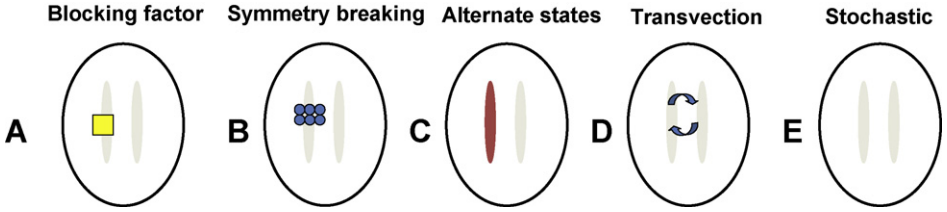

Fig. 1. Different models for initiation of random X inactivation. (A) A blocking factor protects one X chromosome from X inactivation. (B) Accumulation of diffusible molecules on one X chromosome protects it from inactivation. (C) Alternate epigenetic states mark the chromosome that will be inactivated. (D) Both chromosomes meet in space and time determining which one will be inactivated. (E) In a stochastic model each X chromosome has a certain probability of being inactivated.