

Dosage compensation of the X chromosome and Turner syndrome

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Abstract. Turner patients have a karyotype of 45,X, while normal females are 46,XX and normal males are 46,XY. In order to understand Turner syndrome, it is important to understand how gene dosage of the X chromosome is regulated. In this review, we address sex chromosome evolution and the two forms of X chromosome dosage compensation: X upregulation and X inactivation. Recent microarray analyses have provided evidence for two-fold X upregulation in males and females. This equalizes gene dosage between the X chromosome and the autosomes. Inactivation of one of the two X chromosomes in females occurs to prevent functional tetrasomy and to equalize gene dosage between the sexes. However, 15–25% of human X-linked genes escape X inactivation. These escape genes are thought to contribute to the phenotype of Turner patients. Expression of escape genes is tissue-specific, suggesting that their role in Turner phenotypes is tissue-dependent. Recent data support a role for CTCF and chromatin structure in the regulation of genes that escape X inactivation. © 2006 Elsevier B.V. All rights reserved.

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1. Introduction

Normal mammalian females have two X chromosomes, while males have one X and a Y chromosome. Patients with Turner Syndrome (TS) [1] have only one X; their condition results from either the loss of part or all of a second X or Y chromosome. Study of Turner's necessitates the study of how the sex chromosomes evolved and are regulated. There are

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two forms of X chromosome-wide regulation: upregulation and inactivation. X upregulation equalizes gene dosage between the X and the autosomes; however, there are tissue-specific differences in the X chromosome to autosome expression ratio (hereafter designated as X:A expression ratio). X inactivation silences one X chromosome in females and thus equalizes gene dosage between the sexes; however, in females, there are a number of genes that escape inactivation. Elucidating the mechanisms of tissue-specific X upregulation and escape from X inactivation may provide insight into TS.

2. Sex chromosome evolution

The mammalian sex chromosomes evolved from a pair of homomorphic chromosomes [2]. They began to diverge from each other when the male-determining gene, *SRY*, differentiated on the proto-Y chromosome. Recombination with the proto-X chromosome ceased at this locus and eventually led to the loss and differentiation of Y-linked genes. As the proto-Y evolved, genes advantageous for males would have localized near *SRY* to ensure that they would be passed to all male progeny. As this proceeded, the region of non-recombination between the proto-sex chromosomes became larger and larger. Since the proto-Y no longer had a chromosome to recombine with, deletions and mutations would occur. Today, the Y chromosome has lost most of its original genes save *SRY* and some male fertility genes.

The loss and differentiation of Y-linked genes made males haploinsufficient for X-linked genes. Dosage compensation to equalize the expression dosage of all the autosomes and the single X chromosome must have evolved to avoid deleterious effects of monosomy [3]. The predicted form of this dosage compensation would be X upregulation, increasing the expression of genes from the X chromosome two-fold and thus insuring equal gene expression dosage between the X and the autosomes in males. X upregulation has been shown to occur on the single male X chromosome in male *Drosophila*, but it has only recently been shown to occur in mammals [4,5].

3. X upregulation

Our laboratory [4] employed extensive microarray analysis to demonstrate an X:A expression ratio close to one in a variety of mammalian species and tissues (Fig. 1), lending support to the theory that the X chromosome is dosage compensated in regard to the autosomes. The simplest mechanism to achieve this would be to upregulate the expression of the X chromosome by two-fold. It was shown that X upregulation occurs very early in embryogenesis, as haploid germ cells do not have an upregulated X but early embryos do (Fig. 1) [4]. The molecular mechanisms of mammalian X upregulation are presently unknown; in *Drosophila*, a specific protein/RNA complex—the MSL complex—associates with the male X chromosome to modify the chromatin and increase gene expression [6,7]. Whether such epigenetic modifications occur on the mammalian X remains to be determined. Another possibility is that DNA sequence changes took place during evolution to modify regulatory elements of X-linked genes and increase expression.

Another interesting finding from our studies was that the X:A expression ratio was higher (1.1–1.2) in brain than in other tissues [4]. Brain-specific genes have accumulated

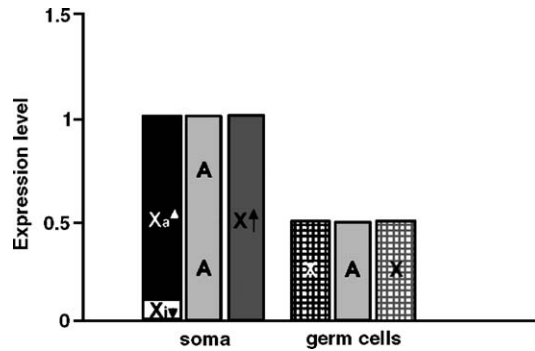


Fig. 1. Dosage compensation in mammals. In the mammalian soma, the expression of each pair of autosomes (light gray) is equal to the expression of the two X chromosomes in females and the single X chromosome in males (arbitrarily set to 1.0). In females, one of the two X chromosomes is active and upregulated (black, up-arrow), while the other X chromosome is inactivated (white, down-arrow). The single X chromosome in males is upregulated (dark gray, up-arrow). In the haploid germ cells of mammals, the X chromosome is active but not upregulated to achieve an equal expression level with the autosomes (0.5; hatched black for female and hatched dark gray for male).

on the mammalian X and are thought to provide a selective advantage in sexual reproduction [8]. In fact, about 40% of X-linked genes are expressed in the brain. X-linked mental disorders are three times more common than other X-linked abnormalities, after taking into account that X-linked inheritance is more easily detected than autosome-linked inheritance [9]. It is estimated that as much as 10% of X-linked genes cause some form of mental retardation when mutated. We did not observe overall differences in the transcriptional output from the X chromosome between males and female brain or non-brain tissues [4]. However, as discussed below, a subset of individual genes, especially escape genes, displayed differential expression between males and females.

4. X inactivation

Mammalian females have two X chromosomes, and thus, X upregulation alone would result in functional tetrasomy. Therefore, females evolved another form of dosage compensation to silence one X chromosome and equalize gene dosage between males and females. X inactivation [10] is initiated early in development. In the female mouse, the paternal X chromosome is inactivated in the 2- to 4-cell stage [11,12]. Extraembryonic tissues continue to display this imprinted paternal X inactivation, while in the embryo, the paternal X is briefly reactivated at the implanted blastocyst stage before one of the two X chromosomes in each cell is randomly inactivated. It is not clear whether this form of early paternal X inactivation occurs in humans; however, random X inactivation becomes established at the blastocyst stage.

The non-coding RNA *Xist* is responsible for the initiation of both imprinted and random X inactivation [13,14]. *Xist* coats the entire X chromosome in cis, triggering a series of epigenetic modifications. These modifications include recruitment of the EED-EZH2 protein complex, methylation of lysine 27 on histone H3 (H3K27), hypoacetylation of H3

and H4, hypomethylation of H3K4, methylation of H3K9, and association of macroH2A [15–17]. Modification of the DNA, i.e., methylation of CpG islands at the 5'-end of X-linked genes, as well as late replication also characterizes the inactive X. These epigenetic changes all contribute to the silencing and maintenance of that silencing of the inactive X. However, not all X-linked genes are silenced by X inactivation. Genes that escape X inactivation play an important role in TS.

5. Escape from X inactivation

About 15–25% of human X-linked genes escape X inactivation (Fig. 2) [18]. These escape genes are expressed from both X chromosomes in the female, although the number of genes that escape and the level of expression vary in different tissues and individuals [19]. The level of expression from the inactive X is often lower, possibly due to different epigenetic modifications of the two chromosomes [18]. Microarray analysis showed a tissue-specific higher expression of escape genes in female mice and humans, although the escape genes contribute little to the overall X:A expression ratio [4]. It is of note that female expression of some escape genes is higher in the adult mouse brain compared to embryonic tissues, suggesting developmental stage related differences [20]. This is important for the understanding of TS phenotypes that occur during development.

Escape genes are expressed from the context of silenced chromatin, the inactive X, and they display epigenetic modifications that are typical of active genes [21]. The localization of actively transcribed escape domains within inactivated chromatin suggests the existence of boundary elements to prevent the spreading of the inactivated chromatin or vice versa.

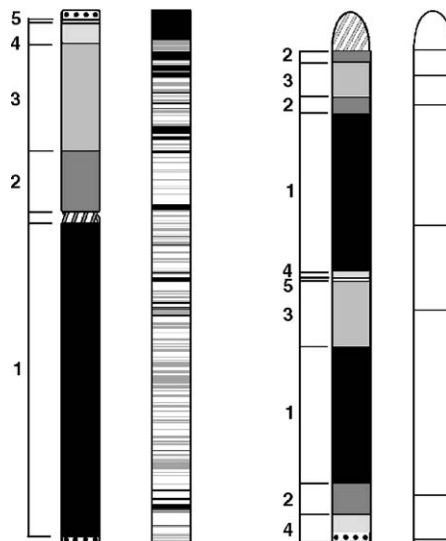


Fig. 2. Distribution of escape genes in human (left) and mouse (right). For each species, the left diagram shows the five evolutionary strata originally defined on the human X [22]; the right diagram displays the distribution of escape genes as horizontal bars [18,21].



Fig. 3. Hypothetical loop model for the insulation of chromatin domains of escape [23]. Chromatin insulator elements including CTCF may mediate the formation of a loop or anchor a domain in a specific nuclear compartment. Escape gene is in gray and silenced genes in black. CTCF are represented as white ovals.

The mammalian chromatin insulator protein CTCF has been found at the boundary between escape genes and inactivated genes [23]. CTCF is hypothesized to prevent DNA methylation and other epigenetic modifications characteristic of silenced chromatin to spread into the escape domains. This is supported by the finding that the CpG island at the 5' end of *Jarid1c* shows a lack of DNA methylation [23]. *Jarid1c* has been shown to be transiently silenced early in development [24], suggesting that it undergoes inactivation along with all X-linked genes and later becomes reactivated due to a lack of maintenance of the silenced state. CTCF has also been hypothesized to mediate the formation of chromatin loops to physically separate the escape domains from the inactivated domains (Fig. 3).

6. Turner syndrome

It is thought that TS is caused by the haploinsufficiency of the escape genes. There are only a few escape genes in the mouse, which may explain why the XO mouse has a near-normal phenotype (Fig. 2) [25,26]. Turner individuals are phenotypically female and suffer from premature ovarian failure and other phenotypes, the most severe being poor viability in utero. TS phenotypes affect a number of tissues (see this issue). TS individuals have normal verbal intelligence but are deficient in visuospatial, arithmetical, and socio-cognitive skills [27]. A majority of the human escape genes are localized on the short arm of the X (Xp), consistent with the findings that most of the TS phenotypes appear to result from a reduced dosage of genes on the Xp, as show by deletion analysis [28]. Examination of X-linked gene expression in multiple adult and embryonic tissues from normal males and females and from Turner individuals will help elucidate the roles of X upregulation, X inactivation, and escape from X inactivation in eliciting the various phenotypes of Turner syndrome.

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