Dosage Compensation*

A Mechanism to Equalize X-linked Gene Products Between the Sexes

Rajiva Raman

The sex chromosomes evolved from a pair of autosomes that deviated over a period of time, with one chromosome losing most of its genes. In many animal groups, females have two X-chromosomes—a large chromosome with numerous genes. Males have one X and a Y chromosome, which has lost most genes except those involved in sex determination and fertility. Thus males are effectively monosomic for the X-chromosome. Monosomy being lethal for other chromosomes, organisms evolved a mechanism called 'dosage compensation' (DC) which quantitatively equalizes X-linked gene products between the sexes, compensating for their numerical disparity (dosage). Best studied in *Drosophila*, *Caenorhabditis elegans*, and mammals, different species adopt different mechanisms of DC. In *Drosophila*, genes on the male X-chromosome are twice as active as on each X-chromosome in females. In *C. elegans*, DC is achieved by the lowered activity of each X-chromosome in XX individuals vis-a-vis the male X. In mammals, the inactivation of an entire X-chromosome in the female results in the parity between the two sexes. Despite the difference in gross mechanisms, the molecular processes achieving DC are uniform due to chromatin modifications (histone acetylation, methylation, and DNA methylation) and synthesis of various noncoding RNAs (lncRNAs). Together, they regulate the X-chromosome activity. In mammals, a lncRNA from the inactive X—*XIST* (X-inactive specific transcript)—binds with the same X to initiate inactivation. X-chromosome inactivation (XCI) in humans reveals interesting mechanisms for en bloc regulation of gene function, as well as modifiers of Mendelian

Rajiva Raman had his university education from Banaras Hindu University. After his retirement from the Department of Zoology at BHU as Professor, he is now serving in the same department as Distinguished Professor. He is also the Senior Scientist of the Indian National Science Academy. He has a teaching experience of nearly 40 years.

Keywords

Dosage compensation, X-linked genes, Barr body, *XIST*, heterochromatisation, X-linked disorders.

*Vol.26, No.5, DOI: https://doi.org/10.1007/s12045-021-1167-3

inheritance patterns in genetic disorders.

Introduction

In most bisexually reproducing animal systems, the sex is chromosomally distinguishable. In mammals and many other groups, for instance, females have two X-chromosomes while males have only one; instead, males have a Y-chromosome that females lack (XX female/XY male). In birds and snakes, females are XY while males are XX (the nomenclature given is ZZ male/ZW female).

In most bisexually reproducing animal systems, the sex is chromosomally distinguishable. In mammals and many other groups, for instance, females have two X-chromosomes while males have only one; instead, males have a Y-chromosome that females lack (XX female/XY male). In birds and snakes, females are XY while males are XX (the nomenclature given is ZZ male/ZW female).

The sex chromosomes are believed to have evolved from a pair of homologous chromosomes that gradually diversified, one of them losing most of its genes and retaining only those essential to the determination of sex and fertility. As a result, while the X-chromosome retains a large number of genes, Y is bereft of them. Thus, the sex with only one X (or Z) may be functionally monosomic for several X-chromosomal genes. Monosomy of a chromosome is generally lethal. The reconciliation of this anomaly concerning the sex chromosomes is the theme of this article.

Dosage Effect – History

As the concept of 'gene' as a physico-chemical entity got accepted, questions regarding its structure, mode of its manifestation into a phenotype, nature of mutations, etc., emerged and intrigued for long. An experiment by the German scientist Curt Stern (1929) on an X-linked mutant 'bobbed' in the fruit fly, *Drosophila*, was instructive. The bobbed flies have shorter than the normal bristles (hairs). Stern generated flies in which he added one more copy of the mutant bobbed locus (3 copies of bobbed but two X-chromosomes). Curiously, the addition of mutant allele led to bristles of normal size, leading Stern to conclude that the 'gene' acts by making a certain product in a measured amount. The amount of the product will depend upon the copy number of the gene, showing the 'dosage effect'. A mutant will alter gene product either in quantity or in quality. The mutant

bobbed allele, Stern explained, made less product than the wild type, hence the shorter bristles. But the addition of another mutant copy added more product that equalized the produce of the 2 normal (wild type) alleles [1].

Dosage Compensation – Concept

Drosophila: In 1932, Herman Joseph Muller, reported an experiment similar to that of Stern using an X-linked eye colour mutant— white^{apricot} (w^{ap})—in which the eye colour is changed from red to patches of red and white like the fruit 'apricot'. The colour of the mutant eyes in both male and female w^{ap} was the same though the female had two mutated X-chromosomes while the male had only one. Addition of one more copy of w^{ap} to the mutant male and female flies (♀X w^{ap} X w^{ap} + w^{ap} /♂X w^{ap} Y + w^{ap}) added more colour to the eye. But whereas the male eye was near normal red, the female had less than the normal eye colour. Addition of one more copy of w^{ap} to the female (X w^{ap} X w^{ap} + w^{ap} w^{ap}) led to normal eye colour. The female required two copies of 'w^{ap}' to produce as much pigment as produced by the single copy in the male (see *Table* 1). Barring a few exceptions (e.g. X^{eosin}), similar results were obtained with most X-linked mutants. Muller concluded that this observation in mutants was also true for wild type genes, and defined it as 'dosage compensation'. He argued that parallel to the evolution of unequal-sized sex chromosomes, evolved the mechanism of dosage compensation (DC) for quantitative equalization of the X-linked products in the two sexes to compensate for the numerical disparity of the X-chromosome between the sexes. DC operates between the sexes, not within a sex where it shows dosage effect (as exemplified by the addition of w^{ap} in female).

However, the concept of dosage compensation was received rather tepidly at the time it was proposed, and for long, no real progress was made in this direction. A resurgence in the study of dosage compensation occurred in *Drosophila* in the early 1960s with two types of studies. John Lucchesi measured the biochemical

Table 1. An illustration of Muller's experiment with W^{a+}(Normal eye) and W^{a} (white apricot eye colour) flies. The horizontal lines depict chromosomes, the longest line is the X-chromosome while that half of its size is the Y-chromosome. Smallest lines show a fragment of chromosome carrying a copy of W^{ap} added to the flies. In the table, first section shows the genotype of different flies, while section 2 shows the phenotype (colour of the eye). The third segment shows the intensity of red pigment in the eyes, the number '+' show greater intensity.)

Genotype/Chromosome Constitution	Phenotype of eye	Intensity of Red pigment in the eye
w^{a+} X (normal male) Y	Red eye	+++
w^{a+} X (normal female) w^{a+} X	Red Eye	+++
w^{a} X (X with white apricot mutation) Y	White with patches of red like apricot	+
w^{a} X (X with white apricot mutation) w^{a} X	white with patches of red like apricot	+
w^{a} X (X with white apricot mutation) $w^{a\,(A}$ (A piece of chromosome with w^{a} Y	Red eye	+++
w^{a} X (X with white apricot mutation) w^{a} w^{a} (A piece of chromosome with w^{a})	More red than W^{a} but not quite the wild type	++
w^{a} X (X with white apricot mutation) w^{a} w^{a} (two pieces of chromosome with w^{a}) w^{a}	Red eye	+++
w^{a+} symbol for the normal eye (Wild type) w^{a} symbol for the mutant - white apricot		

products of the X-linked genes, such as the enzymes glucose-6-phosphate dehydrogenase (G6PD) and phosphoglycerate kinase (PGK). He showed parity in these products between the sexes, confirming dosage compensation in wild type genes. Mukherjee used the technique of cellular autoradiography to study transcription as a measure of global gene function at the chromosomal level in the polytene chromosomes of *Drosophila melanogaster*. He demonstrated that equalization of gene products between the male and female X-chromosomes was achieved by the 'hyper-activation' of the single X-chromosome in male, i.e., the single X-chromosome in the males produce twice as much transcript as by the single X-chromosome in the female [2].

Mammals (Barr Body and Lyon's Hypothesis): In 1949, Murray Barr and his graduate student Bertram, from Canada working on cat neurons, found a chromatin body in the female cells but not in those of males. This observation was soon confirmed

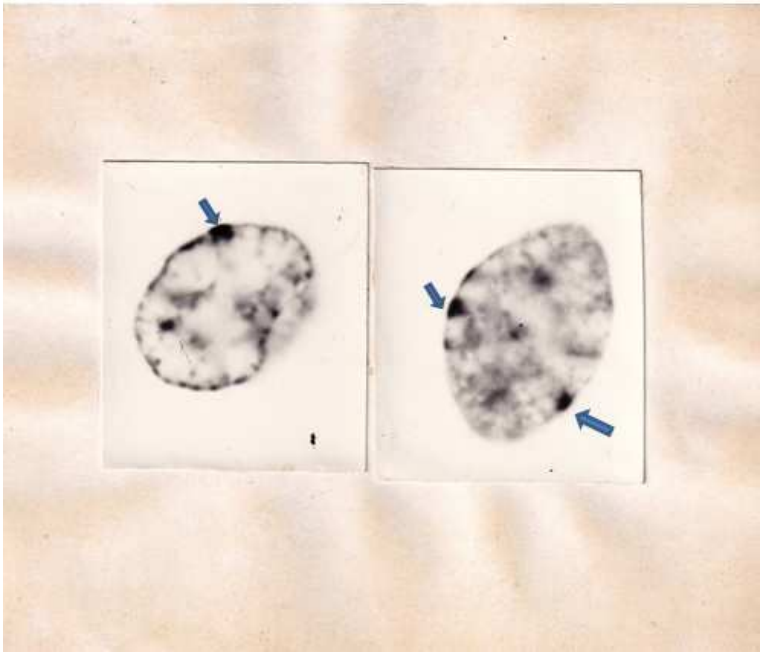

Figure 1. Sex chromatin: The darkly stained body attached to the nuclear periphery (arrows) formed by the single inactive X-chromosome. The nucleus showing single sex chromatin body has two X-chromosomes while that with two bodies would have three X-chromosomes.

in mammals at large, including humans. The sex chromatin is also called 'Barr body' after the name of its discoverer. Later Susumu Ohno (1959) showed that this body comprised a single X-chromosome. Its condensed nature showed that it was heterochromatic[1] (see [3]) (*Figure* 1). Related information followed from human and mouse studies, showing that individuals with only one X-chromosome (X0) were females (though in humans, it would lead to Turner Syndrome), and those with 2 or more Xs along with the Y-chromosome (XXY) would be male, though infertile (Klinefelter syndrome). This evidence while confirming the role of Y-chromosome in male sex determination, also showed that the X-chromosome monosomy was not lethal, as would be expected in the case of autosomal monosomy.

In a different set of genetic experiments on the mouse, British Scientist Mary Lyon found that individuals heterozygous for almost all the X-linked genes for coat colour or hair texture are mosaic with patches of wild and mutant patterns (*Figure* 2). Integrating these cytological and genetic features, Lyon [4] hypothesised that

[1] A state of chromatin that remains condensed through the cell cycle and is genetically nonfunctional.

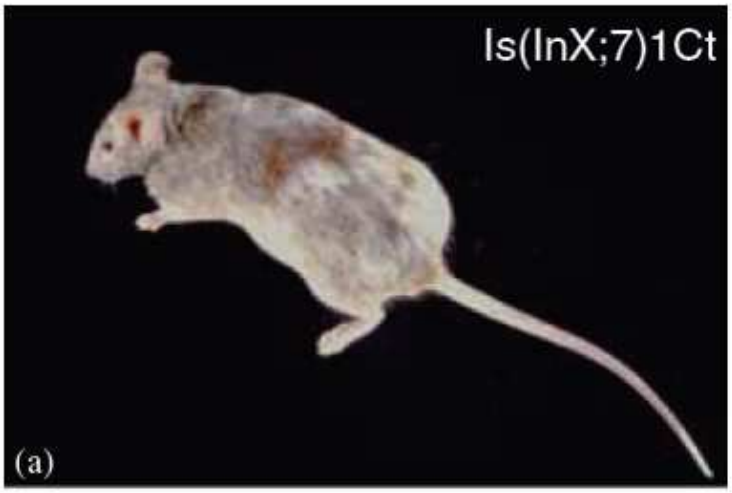

Figure 2. Coat colour mosaicism (brown and white patches) in a female mouse showing spreading effect due to the translocation of a X-chromosome to chromosome 7. (See Disteche and Berletch, 2015 [5])

these features in mammalian females occur due to the inactivation of one of the two X-chromosomes. She elaborated that (1) the entire X-chromosome is inactivated early in development, (2) it occurs randomly in any one of the X-chromosomes in a cell, and (3) X-chromosome inactivation (XCI) is irreversible in all the descendants of that cell. For example, a female, heterozygous for the hair texture gene, *Tabby*, will be a mosaic of large patches of normal hair and patches with tabby texture. Contrast it to an autosomal recessive heterozygote, where the individual will appear normal, not a mosaic. Lyon's hypothesis supported dosage compensation in mammals insofar that both the sexes had only one functional X-chromosome. Ironically, none of these seminal papers, including that of Mary Lyon, mentions the term 'dosage compensation'. A mass of data employing varied genetic, somatic cell genetic, chromosomal, and molecular approaches confirm the main tenets of the hypothesis though exceptions to all its tenets exist [5], which I touch upon later in the article.

Caenorhabditis elegans is an example of a species that has no Y-chromosome and yet is male heterogametic. In *C. elegans*, individuals with one X are males, and those with two Xs are hermaphrodites, which can self-fertilise.

Caenorhabditis elegans: Here is an example of a species that has no Y-chromosome and yet is male heterogametic. *C. elegans* is a soil nematode in which individuals with one X are males, and those with two Xs are hermaphrodites (XO male/XX hermaphrodite), which can self-fertilise. The sex is determined

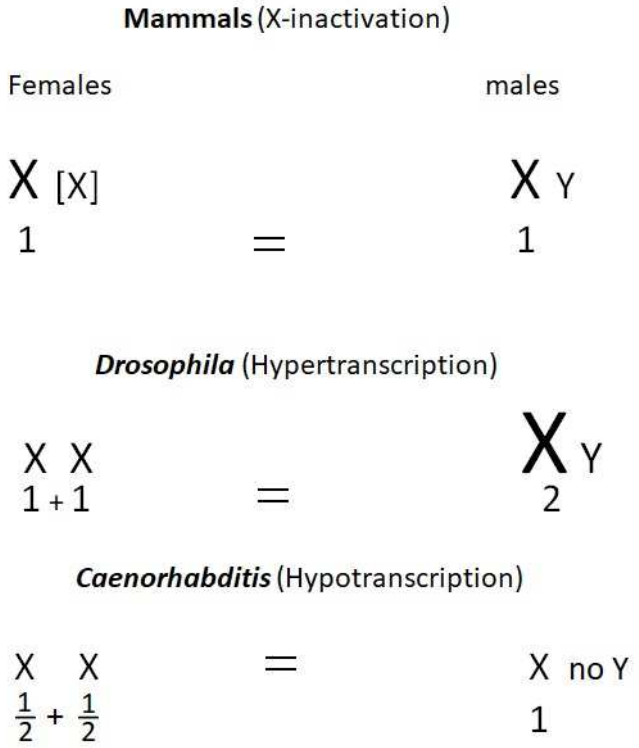

Figure 3. Different strategies to equalize X-chromosome gene product between the sexes. (Modified from the *Current Opinion in Genetics & Development*, 10, pp.644–650, 2000.)

by the number of Xs in an individual. In many insects too, e.g., in grasshoppers, crickets, etc., the male does not have a Y chromosome, but unlike *C. elegans*, XX individuals are females. DC operates in this male heterogametic species too, but through the calibrated lowering of the activity of each X-chromosome in the XX hermaphrodites [6] (see *Figure* 3).

Mechanisms of Dosage Compensation

Genetic and molecular studies have helped delineate the mechanism of dosage compensation. Curiously, different species employ different ways to achieve it, some of which are discussed below.

Genetic and molecular studies have helped delineate the mechanism of dosage compensation. Curiously, different species employ different ways to achieve it.

Drosophila: The sex-determining switch gene Sex-lethal (*SXL*) has antagonistic functions in the two sexes. In the XX embryo,

SXL is active, and its transcript codes for a DNA binding protein, which induces the downstream gene, *tra*, to proceed on the female pathway. In the XY embryo, *SXL* RNA is alternatively spliced, creating a stop codon in the processed RNA, resulting in a truncated, nonfunctional protein. Thus, the absence of the SXL product in the XY embryo triggers the male determining path. In the XX females, SXL additionally silences a group of autosomal genes, called the MSL (Male Sex-lethal) complex, comprising such proteins as ATPase/helicase; E2 ubiquitin ligase and a histone H4 acetyltransferase (HAT) that acetylates lysine in histone H4. In contrast, in the XY embryos, the same MSL complex gets activated because *SXL* is nonfunctional.. In chromatin, DNA and histones are tightly bound because of their opposite charges (DNA –vely charged, Histone +vely charged). However, the addition of an acetyl group (-vely charged) to the histones (such as in Lys16 in histone H4) causes loss of its +ve charge, and thus loosening of its binding with DNA, causing chromatin decondensation and potentiating transcription. The HAT of the MSL complex acetylates the X-chromosome in males only. In addition, the male X-chromosome transcribes two non-coding RNAs, ωX1 and 2 (read as roX), which together with the MSL hyperactivate the X-chromosomal genes in the male. Each of ωX1 and ωX2 has a stretch of sequence called the 'pioneering site' on X (PionX). Initially, MSL binds with any one of the roX RNA through the PionX sequence. This interaction structurally alters the MSL2 subunit leading to a chain of events; MSL2 binds with more sites on the X-chromosome. This binding with multiple X-chromosome sites is facilitated by more proteins and the siRNAs (another group of non-coding RNA) clustered around the transcriptionally active genes. Physical binding of the MSL along the entire X-chromosome enables the HAT of the MSL to acetylate H4 histones on the X-chromosomal genes. This enables their rapid and higher-level transcription, resulting in hyperactivation of the X-chromosome. Thus, a hyperactive male X turns out more product, such that it quantitatively equals the products of the two X-chromosomes in the female.

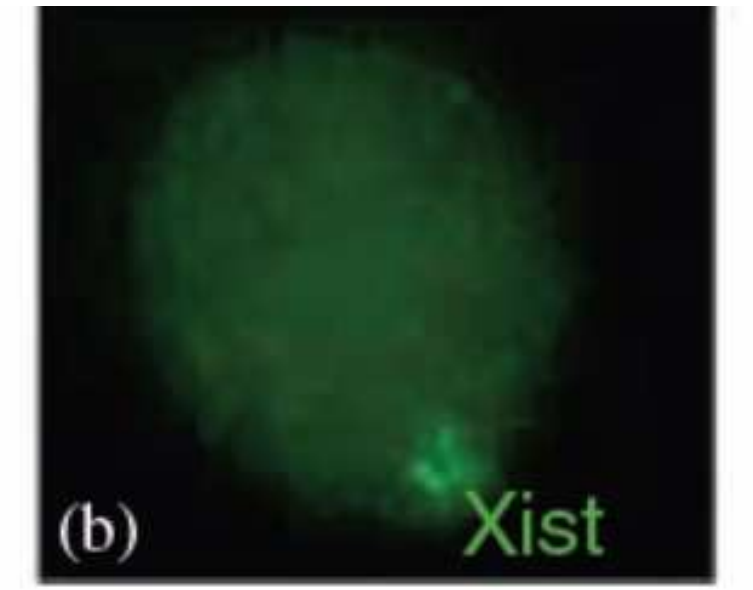

Figure 4. Xist: Nucleus from a female cell from mouse after in situ hybridization with the Xist transcript. Whole X-has been covered by the Xist. (From Disteche and Berletch, 2015 [5].)

Caenorhabiditis elegans: As opposed to *Drosophila*, in *C. elegans*, equalisation of the X-linked products is achieved through lowering of the activity of each X-chromosome in the XX individuals [6]. Though considered hermaphrodite, the XX individuals are essentially females with remnants of the testis to produce male germ cells. The sex-determining system in this species recruits a cascade of genes, each of which, when active, suppresses the expression of its immediate downstream gene, which becomes active in the other sex and in turn represses its downstream gene too. For instance, Xol (mutant male lethal), expresses in the XO (male) and suppresses immediate downstream SDC1, 2, 3 genes. Since Xol is not expressed in XX individuals, the SDC genes are expressed by default kicking start the female pathway by inhibiting immediate downstream her-1 gene. The SDC genes are also involved in dosage compensation (SDC – sex determination and dosage compensation). They become part of a multi-subunit dosage compensation complex (DCC). Hence DCC is present only in the XX individuals. The most important role in this complex is played by the condensin protein that condenses the X-chromosomal chromatin in XX individu-

As opposed to *Drosophila*, in *C. elegans*, equalisation of the X-linked products is achieved through lowering of the activity of each X-chromosome in the XX individuals.

als. As in *Drosophila*, in this species also, chromatin modification takes place at certain X-chromosomal DNA sequence motifs. To these sites (called the recruitment sites; rex sites), DCC initially binds and then spreads through the length of the chromosomes. Methylation of histone protein (H4-K20 — histone H4 lysin at 20th position) adds to the compaction of chromatin and lowering of transcription on the X-chromosomes. Methylation of both DNA and histone is generally associated with the compaction of chromatin. The extent of methylation calibrates the extent of compaction. The DCC complex harbours a demethylase that demethylates H4K20Me2 to H4K20Me1 (dimethyl to monomethyl state). In the mono-methylated state, the X-chromosomal chromatin becomes more compact, lowering the transcription potential of its genes [7].

Mammals: The process of dosage compensation in mammals is different from those seen in the fly and the worm insofar that, unlike the earlier two species, in mammalian female, an entire X-chromosome is inactivated by getting 'facultatively heterochromatised', while the other X remains extended and euchromatic (potentially active). For a comparative view of the different mechanisms, see *Figure* 3.

In mammalian female, an entire X-chromosome is inactivated by getting 'facultatively heterochromatised', while the other X remains extended and euchromatic (potentially active).

In mammals, as earlier stated, Lyon hypothesized that one of the two Xs in the females gets inactivated, and the inactivation occurs randomly and irreversibly in either X, early in development. Though many experiments have validated the propositions of the hypothesis in a large measure, there are exceptions to all its three tenets. (1) While most part of the X-chromosome is inactivated, certain genes in the PAR[2] as well as a few others, escape inactivation, (2) in the prototherian and metatherian mammals and the trophoblast[3] cells in the mouse embryo, only the X received from the father is inactive (non-random), and (3) in the germ cells, the inactive X-chromosome is reverted to an active state before entering meiotic cell division, ensuring that all the gametes (ova/eggs) carry an active X-chromosome. An understanding of the mechanism of X-chromosome inactivation in mammals, therefore, needs to account for the following ques-

[2] A small region in which the X and Y chromosomes share homologous genes.

[3] They make the extra-embryonic tissues.

tions, (i) how does the signal for inactivation spread through the X-chromosome, (ii) what establishes the irreversibility of the inactive X-chromosome, (iii) how do certain genes escape inactivation on the inactive X, (iv) how is the X-chromosome chosen for inactivation in a cell, and (v) the significance of X-inactivation in genetic disorders in humans. We will try to understand the same.

Certain females have one normal X-chromosome, but the other X is fragmented into two, attaching with an autosome due to a reciprocal translocation.

Regulator of X-chromosome Inactivation: Certain females have one normal X-chromosome, but the other X is fragmented into two, attaching with an autosome (karyotype X/X-A, A-X/A; A denotes autosome) due to a reciprocal translocation. In such translocation heterozygotes, the inactive X is predominantly the normal X in almost all the cells, apparently an example of non-random XCI (X-chromosome inactivation). However, during early development in these individuals, both types of cells—those with normal X inactive and translocated X inactive—are present. But those with the translocated X do not survive long because in these cells, only one of the two fragments of the X-chromosome gets inactivated leaving the other one active creating functional disomy of that region. On the other hand, the inactivated fragment spreads inactivation into the contiguous autosome causing functional monosomy of certain autosomal genes (position effect variegation, see *Figure* 2). Such abnormal behaviour leads to the elimination of these cells, and only those cells with the normal X inactive survive. Thus, such cases do not violate the principle of randomness of X-inactivation, it shows instead an example of selection bias.

The above example on the translocated X-chromosome raises the possibility that there will be either one or very few site(s) on the X-chromosome that regulates its inactivation. This presumed regulatory region is called Xce (X-controlling element) in mice and XIC (X-inactivation centre) in humans.

Delineation of Xce (XIC): The molecular delineation of Xce (XIC) was reported in 1991 independently by Brockdorf et al., in mice and Brown et al., in humans [8]. Both the groups obtained a transcript (RNA) transcribed only from the inactive and not from the active X-chromosome. The gene, named *XIST* (X-inactive spe-

cific transcript; *Xist* in mouse), makes 17kb and 14kb transcripts in humans and mice, respectively, which does not translate into a protein.

That *XIST* could be the potential regulator of XCI is drawn from the experiments in which XY cells are transfected with additional copies of *Xist* DNA attached to an autosome or the Y-chromosomes (analogous to those done by Muller in *Drosophila*). The result was the inactivation of either the normal X-chromosome or the autosome (or Y) to which it was attached. Alternatively, in XX cells with one X endowed with a loss-of-function mutation of *Xist*, despite the presence of two X's, no XCI is seen. These experiments confirmed the role of *Xist* in initiating X-inactivation. But they also demonstrated the role of *Xist* as a numerator of the X-chromosomes in a cell. In other words, the number of *Xist* in a cell would practically equate with the number of X-chromosomes.

In *Drosophila*, hyperactivation of the X-chromosome is mediated by the loosening of the DNA-histone binding through acetylation of H4 histone, and in *C. elegans*, it is the methylation of Lys 20 in H4 that leads to the compaction of chromatin.

Xist binding, Chromatin Modification and Heterochromatisation of the X-chromosome: The 17/14kb Xist RNA spreads on the same X-chromosome from which it is synthesized, binding to several sites through its entire length, except the PAR. Once wrapped with Xist (*Figure* 4), several other chromatin-modifying factors act on the X-chromosome and heterochromatinise it. We have mentioned that in *Drosophila*, hyperactivation of the X-chromosome is mediated by the loosening of the DNA-histone binding through acetylation of H4 histone, and in *C. elegans*, it is the methylation of Lys 20 in H4 that leads to the compaction of chromatin. In mammals too, modification of histone plays a crucial role in XCI. A modified histone called MacroH2A[4] is selectively present on the inactive X-chromosome on many sites along the chromosome. In addition, histone modifications on specific sites are not only distributed in the coding and promoter regions of the genes but also in the intergenic regions of the inactive X-chromosome. The trimethylation of lysine at the 27th positon (K27me3) and dimethylation of K9 (K9me2), both on H3 histone, in the coding regions on the X-chromosome are the biomarkers of XCI due to chromatin compaction. *XIST* binding of the

[4] A form of histone H2A.

X-chromosome precedes histone modifications and thus may be causal in initiating the XCI. Having initiated the X-inactivation through histone modifications, *Xist* is silenced in subsequent cell cycles in most cell types. Thus the critical function of *Xist* lies in (1) counting the number of the X-chromosomes in a cell vis-à-vis the sets of autosomes (one active X per diploid genome, e.g. 2X/2A – one X inactive; 3X/2A – two X inactive; tetraploid genome 4X/4A – 2 X inactive), and (2) selection of the chromosome for inactivation by binding with it and initiating a process to make it compact and heterochromatic for inactivation.

Irreversibility of X-chromosome Inactivation: The initiation of XCI requires an additional layer of regulation to achieve irreversibility of inactivation on the same X-chromosome in all the descendent cells. That is, the daughter cells after every cell division should remember which of the two X-chromosomes was inactive in the mother cell. Riggs, in 1976, had opined that the mechanism underlying XCI could be through the modification of DNA brought about by the post-replication methylation of the nucleotide, cytosine. It soon became clear that methylation of genes is an important regulator of gene function, often leading to their repression. Subsequent studies also showed that most of the genes on the inactive X were highly methylated as compared to their homologues on the active X-chromosome. It was also experimentally demonstrated that under appropriate selection conditions, the genes on the inactive X-chromosome could be reversed to activity by demethylating them.

It was also experimentally demonstrated that under appropriate selection conditions, the genes on the inactive X-chromosome could be reversed to activity by demethylating them.

DNA methylation also explains the irreversibility mechanism of the inactive X-chromosome. In higher organisms, post-replicational addition of a methyl group ($-CH_3$) at the 5th position of cytidine is perpetuated through subsequent cell cycles if the methylated C occurs as a doublet of CpG so that the complementary strand too has CpG on that site. Methylation is brought about by the enzymes called DNA methyltransferase (MTase), which belong to two broad categories, *de novo* DNA MTase and maintenance DNA MTase. During early development, unmethylated CpG doublets, are methylated on both the strands, at the same time, by

de novo MTase. However, after each replication cycle, the new DNA becomes hemimethylated because the 'C' on the new strand is unmethylated. The maintenance DNA MTase in the cell, if present, identifies the hemimethylated cytosines of the new DNA molecule and methylates the cytosine on the new strand, restoring its status of complete methylation. Thus once methylated, the same sites are remethylated after every replication cycle, and the same X continues to remain inactive in subsequent cell generations. DNA methylation essentially induces chromatin compaction and gene repression by invoking other chromatin modifiers such as histone methylases and MeCPs (methylated cytosine binding proteins). Thus histone and DNA modifications not only inactivate the chromosome but also ensure the irreversibility on the chromosome.

One of the tenets of XCI is the random choice of the X-chromosome in each cell. This question has vexed scientists for long, and a satisfactory answer is still not in sight.

Choice of the Inactive X-chromosome in a Cell: One of the tenets of XCI is the random choice of the X-chromosome in each cell. This question has vexed scientists for long, and a satisfactory answer is still not in sight. Nevertheless, there is some clarity on the way *XIST* expression is regulated during early development. Although there is a difference in the expression pattern of *XIST* in different species, the information we discuss here is mainly derived from humans and mice. In early human embryonic development, both the X-chromosomes tend to be active and *XIST* also is expressed from both of them, though in small quantity and with rapid degradation. Experiments on induced embryonic stem cells (ESC) from humans have confirmed that certain genes are active on both the X-chromosomes during early embryonic development. As differentiation sets in, a large number of different factors, mainly non-coding RNAs, bind with *XIST* and its transcript to potentiate (or suppress) its XCI function, which means that there are factors that induce its function just as there are others which block its expression. The activation of *Xist* in mice is prompted by two other lncRNA genes, JPx and Ftx, located a few kbs upstream of *Xist*. *XACT* is another 250kb lncRNA, made from a gene 40kb away from Xic. Both *XIST* and *XACT* genes are activated around the same time and initially bind to both the

X-chromosomes though at different motifs. But later, when *XIST* stabilizes on one X-chromosome and binds with it to inactivate it, the *XACT*-bound X-chromosome becomes the active X because it inhibits *XIST* to act upon it. However, the main repression of *XIST* is brought about by another gene *TSIX*, residing at the 3′ end of *XIST* gene itself on the opposite strand of DNA. Its transcript runs across the *XIST* gene in the opposite direction, making an antisense RNA that could bind with the *XIST* or its transcript and block it. Thus we have some idea about how the expression and repression of the *XIST* gene on the active and inactive X-chromosomes are regulated and the role of hitherto neglected non-coding RNAs in this process. However, it does not expressly answer what decides which X will become inactive in a given cell. There is evidence that before being selected to their functional fate, both the X-chromosomes briefly touch each other, but what transpires at that stage is not clear. Since several molecules bind with the *XIST* gene, a stochastic model envisages that random excess or competitive binding of one or the other group of factors on an X-chromosome would attract more of its kind on that X and seal its functional fate. There would also be a mechanism that would ensure that once *XIST* is chosen to be active on one X-chromosome for XCI, it is blocked from getting active on other X-chromosomes in the cell.

X-Chromosome Inactivation and X-linked Disorders

An evidence that enabled Lyon to formulate her hypothesis was the fact that individuals with the loss of an X-chromosome were viable both in mice (2n 39, X) and humans (45, X). It was particularly striking because monosomy of no other chromosome, however small or gene-poor, is viable in humans or mice. This evidence hinted that one of the two X's in female is redundant. However, 45, X women suffer from Turner syndrome (*Figure* 5). They are infertile and short-statured with foot edema, etc. The loss of even a part of the X-chromosome, especially the short arm (Xp) causes Turner syndrome. The Xp is endowed with genes in the pseudoautosomal region (PAR), which escape inactivation,

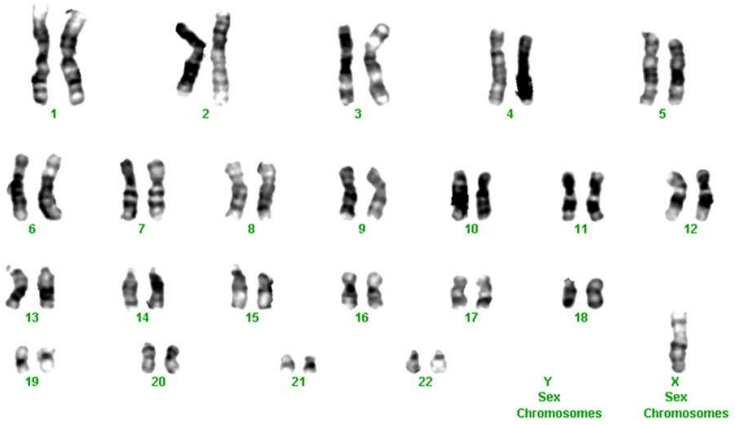

Figure 5. Turner Syndrome: Human karyotype showing 45 chromosomes with a single X-chromosome. Prior to staining with Giemsa stain, chromosome preparation was treated with dilute trypsin solution, which induced G-banding that enables precise identification of each chromosome. (From Amit K. Rai, Centre for Genetic Disorders, Banaras Hindu University.)

and are active on both the X-chromosomes. X-linked form of the Kallmann syndrome is caused due to loss of a small part of its short arm (Xp22.3), which harbors *Kal-1* gene (*Figure* 6). Similarly, X-linked ichthyosis[5] in female is caused by the loss or mutation in the steroid sulfatase (*STS*) gene, even though a normal allele is present on the other X. Since these genes are active on both the Xs, loss of one copy manifests as disease in females, though not as severely as in the males.

[5] A disease of the skin.

Haemophilia A, colour blindness, Lesch–Nyhan syndrome, Duchenne muscular dystrophy (DMD) are all examples of X-linked recessive disorders which generally follow the expected rules of inheritance and affects males.

As per the Mendelian inheritance pattern, in the recessive autosomal mutations, the carriers (heterozygotes) appear normal because in all its cells the product made by the normal allele is able to make up for the mutant allele. The heterozygosity of an X-linked gene in females generally goes undetected because they are expected to be normal as in autosomal carriers.

Haemophilia A, colour blindness, Lesch–Nyhan syndrome, Duchenne muscular dystrophy (DMD) are all examples of X-linked recessive disorders which generally follow the expected rules of inheritance and affects males. However, in all these cases, a certain proportion of the females also manifests the mutant phenotype. It is because, unlike the autosomal heterozygotes where both the homologues are functionally equivalent, in the case of X-chromosomes, in each cell only one X is active, and in around an equal proportion of cells either of them is inactivated. The X-

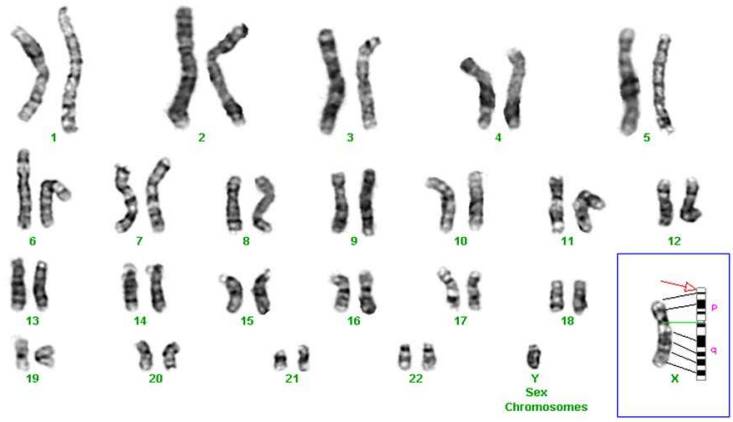

Figure 6. Kallmann Syndrome: Human karyotype showing 46, XY chromosomes with a deletion in the short arm of the X chromosome (Xp22). Kallmann is more common in males and manifests rarely in females. (From Amit K. Rai, Centre for Genetic Disorders, Banaras Hindu University.)

linked heterozygotes, therefore, are a mosaic of cells that show normal phenotype (X-chromosome with the mutant allele inactive) and those showing the mutant phenotype (normal allele-X inactive). Since these cells occur in equal proportion in all tissue types, the overall phenotype appears normal and unaffected. However, if the ratio between the two cell types gets skewed, it can affect the phenotype. For instance, as discussed above in females with an X-autosome translocation, it is the normal intact X (not the translocated one) that gets inactivated in almost all the cells. In these females, if the mutant gene is present on the translocated, active X, then the normal allele will not express at all, and the individual will have the disease, despite being a heterozygote. In DMD for instance, the causal gene dystrophin, which is the largest human gene (>2Mb) is often fragmented and is part of a reciprocal translocation. The skewed ratio of the inactive X due to translocation is often the cause of DMD in women. The skewing of ratio may occur due to a variety of reasons, e.g., a mutation in the *XIST* gene in an X-chromosome would insulate that chromosome from getting inactivated and only the other X, with normal *XIST*, will be inactivated. There is another example of an X-linked disorder, Rett syndrome, which is seen only in females. In this case, a heterozygote for mutation in the X-linked gene MeCP2 (the protein that binds to methylated DNA and facilitates its condensation) leads to severe neural disorders

In females with an X-autosome translocation, it is the normal intact X (not the translocated one) that gets inactivated in almost all the cells. In these females, if the mutant gene is present on the translocated, active X, then the normal allele will not express at all, and the individual will have the disease, despite being a heterozygote.

in females. The mutant males possibly die in utero. It is thus an example of an X-linked dominant disorder, and that this gene, present on the long arm of the X-chromosome (Xq), and thus away from the PAR, is active on both the X-chromosomes. Since MeCP2 is an important component of the DNA-methylation induced condensation of chromatin, its loss of function may affect the regulation of the function of many genes. Apart from these Mendelian disorders, the clonal nature of XCI comes in handy in identifying the original source of cancers.

Thus, the presence of an inactive X-chromosome in females has a specific bearing on the manifestation of X-linked disorders, which becomes an important facet in medical genetics in delineating the molecular etiology of several disorders.

Concluding Remarks

The phenomenon of dosage compensation is a unique example of differential gene regulation as a function of sex and sex chromosomes.

The phenomenon of dosage compensation is a unique example of differential gene regulation as a function of sex and sex chromosomes. In this review, I have tried to summarise pieces of evidence that support the existence of this mechanism in male heterogamety systems. As a postgraduate student, in 1969, when I was first exposed to the concept of XCI, I was struck with many questions right in the middle of the lecture. Today, fifty years later, when so much more has been learnt about this phenomenon, many more questions arise. For instance, how is the X-chromosome chosen in each cell, is there indeed functional monosomy of the X-chromosome, and if so, how is it accepted when monosomy of no other chromosome is tolerated, why is the in utero death rate of 45X individuals as high as 90%, and whether or not dosage compensation operates in the female heterogamety (XX male/XY females) species such as birds, snakes, butterflies, honey bees, etc. The questions posed here are not new, nor untackled; active work is being done on these and many other questions not spelt here. A vibrant topic engenders more questions and novel approaches to tackle them. If this article motivates a few of you to generate questions related to the phenomenon and compels you to think of

ways to answer them, you are already on the right path.

Acknowledgement

I record my deep sense of appreciation to those tens of hundreds of students, whom I have had the privilege of teaching in classrooms and who have responded with interest, curiosity, and innovative ideas. I am deeply thankful to Prof. Vidynanda Nanjundiah, IISc, Bangalore, Dr Indrajit Nanda, Wurzburg, Germany, and Prof. Mahesh Sharma, M.S. College, Saharanpur, Meerut University, for critical appraisal of the manuscript in its formative stage. Springer is thanked for the permission to reproduce *Figures* 2 & 3 from *J. Genetics*. The karyotypes are of the cases studied in the Centre for Genetic Disorders, Banaras Hindu University.

Box 1. Explanation of Certain Terms

Monosomy: In diploid cells, each chromosome occurs in a pair. Loss of one of a pair of a chromosome leads to monosomy in a cell, tissue or individual. Monosomy in an individual is generally lethal.

Phenotype: The morphological manifestation of the genotype is the phenotype of an individual, which is generated through the interaction between the genotype and the environment.

Reciprocal Translocation: An exchange between two chromosomes due to a break in both and then the broken ends of each, fusing with each other. In a heterozygous individual, besides having one intact copy of each of the chromosomes, cells would have two recombined chromosomes due to reciprocal fusion of the broken ends. In an X-autosome translocation, the inactive X-chromosome is predominantly the normal X-chromosome.

Facultative Heterochromatisation: It is a state of chromatin under which a chromosome, or part of it, gets condensed and heterochromatised, while its homologue remains euchromatic.Inactivation of X-chromosome in mammalian females is a good example where one of the two identical X-chromosomes, becomes heterochromatic.

Prototherians and Metatherians: In eutherians (in class Mammalia), the entire development of the embryo occurs in the uterus of the mother and a new individual is born, but there are two more primitive groups of mammals. The most primitive are the prototherians which lay eggs (e.g., duck-bill mole) and have ill-defined sex chromosomes, which may not show XCI. In metatherians, the embryo grows until the advanced stage in the womb of the mother, but before the completion of the development is laid out into a maternal pouch in which it grows till it fully matures (e.g., Kangaroos). In metatherians, the inactive X-chromosome is invariably the paternal X. That is, paternal X is imprinted to be the inactive one in the female.

Trophectoderm: In mammals, very early in development, the embryo is partitioned into two groups of cells called the trophectoderm and the inner cell mass. While the latter forms the embryo proper, trophectoderm, made up of trophoblasts, differentiates into extra-embryonic membranes which nurse the embryo (ICM) by supplying the maternal nutrients to it. In mouse, the inactive X-chromosome in the trophoblasts is invariably the paternal X, as in metatherians.

Position Effect Variegation: First shown in *Drosophila*, if a euchromatic region is brought into the immediate vicinity of the heterochromatin, it gets heterochromatised to a variable degree. Thus the property of chromatin may alter depending upon the position it is placed. This property of chromatin is called position effect variegation or spreading effect.

Non-coding RNA: RNA is generally perceived as an intermediary, which carries the message from the genetic material (DNA) and its final product protein by carrying the triplet codes for the amino acids. The past 20–30 years of research has brought in a long list of RNAs that do not carry an open reading frame (ORF) to code for a protein, instead, they act on their own as regulators of gene function. *XIST*, *TSIX*, *XACT*, *roX* are all examples of non-coding RNAs that regulate dosage compensation in different species.

Suggested Reading

[1] Curt Stern, Dosage compensation: Development of a concept and new facts, *Can. J. Genet. Cytol*., Vol.2, pp.105–118, 1960.

[2] Ardhendu S Mukherjee, Wolfgang Beermann, Synthesis of ribonucleic acid by the X-chromosome of *Drosophila melnogaster* and the problem of dosage compensation, *Nature*, Vol.207, pp.785–786, 1965.

[3] Ursula Mittwoch, Sex chromatin, *J. Med. Genet*., Vol.1, pp.50–76, 1964.

[4] Mary F Lyon, Gene action in the X-chromosome of the mouse (*Mus musculus L*), *Nature*, Vol.190, pp.372–373, 1961.

[5] Christine M Disteche, Joel B Berletch, X-chromosome inactivation and escape, *J. Genet*., Vol.94, No.4, pp.591–599, 2015.

[6] Barbara J Meyer, L P Casson, *Caenorhabditis elegans* compensates for the difference in X-chromosome dosage between the sexes by regulating transcript levels, *Cell*, Vol.47, pp.871–881, 1986.

[7] Sarah Elizabeth Albritton, Sevinc Ercan, *Caenorhabditis elegans* dosage compensation: Insights into condensin-mediated gene regulation, *Trends in Genetics*, Vol.34, pp.41–53, 2018.

[8] Catherine Patrat, Jean-Francois Ouimette, Claire Rougeulle, X-chromosome inactivation in human development, *Development*, doi 10,1242/dev.183095 2020.

[9] John Lucchesi Transcriptional modulation of entire chromosomes: dosage compensation, *J. Genet*., Vol.97, pp.257–364, 2018.

[10] Murray L Barr, E G Bertram, A morphological distinction of the male and female, and the behavior of the nucleolar satellite during accelerated nucleoprotein synthesis, *Nature*, Vol.163, p.676, 1949.

Address for Correspondence
Rajiva Raman
Cytogenetics Laboratory
Department of Zoology
Banaras Hindu Univesity
Varanasi 221 005, India.
Email:
rajiva.raman@gmail.com