X-chromosome Inactivation

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Based in part on the previous version of this Encyclopedia of Life Sciences (ELS) article, X-chromosome Inactivation by Matthew J Wakefield and Jennifer AM Graves

Advanced article

Online posting date: 30th April 2008

X-chromosome inactivation is an epigenetic process that silences the majority of genes on one of the two X-chromosomes in female mammals. This silencing effectively equalizes dosage of X-linked genes in females with that of males, who only possess one X-chromosome and a sex-determining Y-chromosome.

X-chromosome Inactivation

X-chromosome inactivation (XCI) turns off the majority of the genes on one of the two X-chromosomes in females, a unique example of large-scale gene regulation. Mammals have an XX female:XY male chromosome sex determination system that is based on a male-determining Y-chromosome. The X is large and contains approximately 1000 genes. By contrast, the Y-chromosome is a small, mainly heterochromatic chromosome that contains few genes other than the testis-determining factor and spermatogenesis genes. The X and Y pair over only a small region of homology and share about 14 genes in the nonrecombining region. Thus, most genes on the X have no partner on the Y and are present in only one copy in males. These heteromorphic sex chromosomes present an obvious difference in gene dosage. It is apparent from the phenotype of trisomies such as Down syndrome that the dosage of at least some genes is finely balanced and is critical for normal development and function. It is therefore not surprising that mechanisms have evolved to compensate for the differences in genetic dosage caused by sex chromosome heteromorphy. The importance of this dosage compensation system is underlined by the observation that mutations that disrupt the inactivation system are lethal early in female development.

ELS subject area: Genetics and Molecular Biology

How to cite:
Hore, Timothy A; Wakefield, Matthew J; and, Graves, Jennifer AM (April 2008) X-chromosome Inactivation. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester.
DOI: 10.1002/9780470015902.a0005795.pub2

Lyon Hypothesis

XCI was first proposed by Mary Lyon (1961). Her hypothesis was based on the observations that several X-linked, mutated, coat colour genes result in a mosaic phenotype consisting of patches of mutant colour and wild-type colour in heterozygous female mice. Lyon proposed that one of the X-chromosomes was randomly inactivated in each somatic cell of a female embryo early in development. This inactive state is then stably inherited, resulting in patches of mutant and wild-type cells (**Figure 1**). The sizes and shapes of the patches depend on the migration of cells and the proportion of cells in which the normal and mutant alleles are active, and vary between individuals by chance. Since mosaicism is also apparent for coat colour in tortoiseshell cats and in the eyes of human patients with sex-linked ocular albinism, as well as other genes with localized action, Lyon correctly predicted that XCI is a general phenomenon occurring in mammals.

The Lyon hypothesis was supported by observations that one X-chromosome was condensed into a heteropyknotic sex chromatin body, termed the Barr body. The inactive X-chromosome was found to replicate later than the active X and, combined with the heterochromatinization of the X, indicated that inactivation is a whole-chromosome effect (Lyon, 1962).

Molecular Basis of X-chromosome Inactivation

The inactivation of the X-chromosome in mammals results from transcriptional repression. It is a complex, multistep process that involves changes in chromosome conformation, late deoxyribonucleic acid (DNA) replication, DNA methylation, nonprotein-coding ribonucleic acids (RNAs), histone modification and accumulation and exclusion of specific histone isoforms.

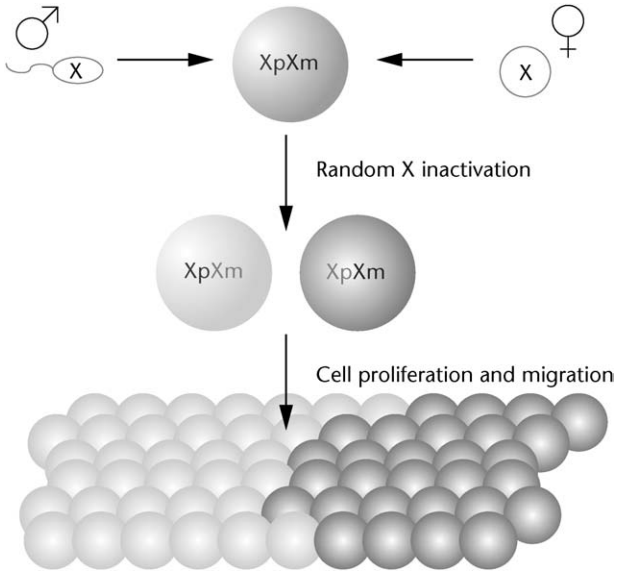

Figure 1 In female cells one X-chromosome is inherited from each parent. Random inactivation means that in some cells the paternally inherited X-chromosome is inactivated, while in other cells the maternal X is inactivated. Cell proliferation and migration continue after inactivation and results in clumps of cells with either the paternal or maternal X inactivated. When the X-chromosomes have different alleles this is apparent as clumps of cells with a different phenotype, such as the coat pattern in tortoiseshell cats.

Although not yet fully understood, XCI has been extensively studied in humans and mice. Classic cytogenetic studies in these species showed that the initiation of XCI is controlled by a 1-Mb domain located just below the centromere called the X-inactivation centre (XIC) (Rastan, 1983).

Initiation

Initiation of XCI involves two critical mechanisms. An X-chromosome counting mechanism is needed to sense the number of Xs in the cell, at least two of which are required for inactivation to occur. The second mechanism must choose (usually at random) which X-chromosome to inactivate. Before the initiation of XCI the two XICs transiently colocalize (Xu *et al*., 2006; Bacher *et al*., 2006). It is proposed that during this time X-chromosome counting and choice occurs. *Xist* (for X-inactive specific transcript) is a large noncoding RNA central to the XIC and is essential for XCI (Penny *et al*., 1996). Transgene and knockout studies in mice show that multiple elements 3′ of *Xist* are responsible for counting and choice, including the noncoding transcripts, *Tsix* (antisense to *Xist*) and *Xite* (X-intergenic transcriptional element) (reviewed Heard and Disteche, 2006). It is currently debated whether the orthologous region of human has a similar function.

Spreading

X inactivation spreads out from the XIC along the entire X-chromosome. *XIST* RNA is believed to have a major role in this spreading, as it coats the entire inactive X-chromosome, the only chromosome from which it is expressed. The mechanism of this spreading, and the means by which *XIST* RNA stays associated with the X-chromosome, is not yet understood. It has been speculated that long interspersed nuclear element 1 (LINE1) repeat elements, which are enriched on the X-chromsome, may act as 'waystations' assisting in *XIST* RNA localization and spreading along the inactive X. This theory is supported by the limited spread of *XIST* RNA into autosome regions that are translocated onto the X-chromosome (reviewed Lyon, 2006).

The mechanism by which *XIST* induces gene silencing once associated with the inactive X is currently under active research. The first known event occurring after *XIST* accumulation is the formation of a transcriptionally silent compartment within the cell nucleus (Chaumeil *et al*., 2006). Genes destined for inactivation are translocated towards the centre of this repressive compartment with assistance from the *XIST* 'A-repeats'. These repeats consist of a tandem array of highly conserved double stem loop structures located near the start of the *XIST* RNA and are essential for silencing (Wutz *et al*., 2002). Coincident with gene silencing is a series of histone modifications on the inactive X, including deacetylation, ubiquitination, alteration in methylation patterns and use of histone variants (reviewed Heard and Disteche, 2006). Sometime after these changes have occurred, XCI becomes irreversible and independent of *XIST* expression (Csankovszki *et al*., 1999).

In humans and mice, XCI appears to be stabilized by the methylation of cytosine residues at clusters of cytosine phosphate guanine (CpG) sites in the promotor regions of genes on the inactive X. Treatment with a demethylating agent was shown to reactivate the inactive X in rodent–human somatic cell hybrids (Graves, 1982). Likewise, knockout of DNA methyltransferase 1 (*Dnmt1*) causes reactivation of genes on the inactive X from the embryo proper, but interestingly, not from extraembryonic tissues (Sado *et al*., 2000).

Late replication

One of the most consistent features of the inactive X-chromosome is that it replicates later in the cell cycle than the active X-chromosome and the autosomes. The mechanism of this late replication and its role in the X-inactivation process are unknown. Late replication may be merely a consequence of a less-open chromatin structure or the spatial isolation of the inactive X-chromosome in the nucleus; however, it may be important in maintaining the differential features of the inactive X-chromosome.

Nonrandom X-chromosome Inactivation

Although most XCI is random, there are several cases where inactivation is nonrandom.

Paternal inactivation

In the extraembryonic tissues of rodents and cow, XCI is imprinted so that the paternal X-chromosome is always inactivated. This may reflect differences in timing, as extraembryonic tissues are the first to differentiate in the developing embryo, possibly before the random choice mechanism for determining XCI is active.

Skewing

The ratio of cells with paternal and maternal inactive X-chromosomes is not always 1:1 in random X inactivation. This can be caused by variation at the gene locus that controls X inactivation biasing the choice of the chromosome to be inactivated. The controlling element was initially discovered and genetically mapped using interstrain variation in the extent of coat colour variegation (Cattanach and Isaacson, 1967). In humans, a mutation in the promotor of *XIST* has been identified that causes a complete biasing of inactivation (Plenge *et al*., 1997).

Apparent nonrandom X inactivation commonly results from selection against cells heterozygous for a recessive mutation when the only functional allele is inactivated. When the mutation is in a tissue-specific gene, skewing may be seen in one tissue and not others. Skewing of X inactivation also occurs when autosomes are translocated onto the X-chromosome, as spreading of inactivation into the autosomal segment is nearly always deleterious (Migeon, 1998).

Genes That Escape X-chromosome Inactivation

Although XCI is a whole-chromosome phenomenon, it is not complete. Several genes on the human X show partial or complete escape from X inactivation. As a consequence, females with Turners syndrome (XO) have a distinct phenotype, presumably caused by the loss of expression of X-inactivation escapees. One class of these exempt genes are located in the pseudoautosomal region of the X-chromosome, which pairs and recombines with the Y at meiosis. This region is homologous between the X- and Y-chromosomes, so it is present in two copies in males as well as females, and dosage compensation is unnecessary. The necessity for dosage equivalence may have provided pressure for the evolution of boundary elements or other mechanisms to prevent the spread of XCI into this region.

Around 15% of genes from outside the pseudoautosomal region also escape XCI on the human X (Carrel and Willard, 2005). A small amount of these genes retain active homologues within the male-specific portion of the Y-chromosome and may not need dosage compensation. It is thought that many of the other escapees lost their Y partner only recently, so that selective pressure has not yet been strong enough to induce compensatory changes to its dosage by inclusion in the X-inactivation system (Graves, 1998). This is supported by the clustering of most human genes that escape inactivation to a regions like the short arm of the X (Carrel and Willard, 2005), which have been added recently to the X-chromosome (Graves, 1995).

X-chromosome Inactivation in Marsupials

Genome sequencing has revealed that *XIST* orthologues are widespread amongst eutherians ('placental' mammals like humans), the largest of all mammalian clades (Hore *et al*., 2007). This indicates that all eutherians probably share an XCI system much like the characterized systems of human and mouse. Marsupials (pouched mammals) also possess XCI, but it is different to XCI of eutherians. First, marsupials inactivate the paternally derived X-chromosome in all tissues, not just the extraembryonic tissues as in rodents and cow (Richardson *et al*., 1971). Although the marsupial inactive X shares late replication (Graves, 1967) and some histone modifications (Wakefield *et al*., 1997) with the inactive X humans and mice, marsupials do not possess an *XIST* orthologue (Duret *et al*., 2006; Hore *et al*., 2007), hypermethylation of gene promoters on the inactive X (reviewed Cooper *et al*., 1993) or an obvious Barr body (McKay *et al*., 1987). The extent with which marsupial and eutherian XCI systems share a common evolutionary origin is currently debated.

Conclusion

XCI is a complex, multistep process involving noncoding RNAs, chromatin remodelling and DNA modification in a way that is still poorly understood. Ongoing research into this process promises to illuminate the evolution and control of XCI and its implications for basic mechanisms of gene regulation across the genome. **See also**: Chromosome X: General Features; Dosage Compensation Mechanisms: Evolution; Epigenetic Factors and Chromosome Organization; Sex Chromosomes; X and Y Chromosomes: Homologous Regions; X-chromosome Inactivation and Disease; Long Interspersed Nuclear Elements (LINEs)

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Web Links

X(inactive)-specific transcript, antisense (TSIX); MIM number: 300181. OMIM: http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id = 300181
X(inactive)-specific transcript (XIST); MIM number: 314670. OMIM: http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id = 314670