International Journal of Micro Biology, Genetics and Monocular Biology Research
Vol.7, No.1, pp.1-20, 2024
Print ISSN: ISSN 2059-9609
Online ISSN: ISSN 2059-9617
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

Animal Breeding and Genetics Specialization: Review on Mechanisms of X-chromosome inactivation in different mammals

Chalachew Mesfin

doi:https://doi.org/10.37745/ijmgmr.15v7n1120 Published January 14, 2024

Citation: Mesfin C. (2024) Animal Breeding and Genetics Specialization: Review on Mechanisms of X-chromosome inactivation in different mammals, *International Journal of Micro Biology, Genetics and Monocular Biology Research*, Vol.7, No.1, pp.1-20,

ABSTRACT: *Sex chromosomes differ significantly in their gene content (Adler et al., 1997), which has led to the evolution of mechanisms of dosage compensation (LYON, 1962; Mary F. Lyon, 1961). At least three different mechanisms have been described to adjust sex chromosome gene expression dosage between males and females. The single male X-chromosome is hyper transcribed in flies; both X-chromosomes are partially repressed in worms, while in mammals one of the two X-chromosomes is silenced in each cell (Mary F. Lyon, 1961). In mammals, dosage compensation for X-linked gene products between the sexes is achieved by X-chromosome inactivation (XCI) in females (Mary F. Lyon, 1961). This process leads to the highly regulated transcriptional silencing of one of the two X-chromosome during early development, leading to the formation of the heterochromatic Barr body.*

KEYWORDS: animal breeding, genetics specialization, mechanisms of X-chromosome, different mammals

INTRODUCTION

X- Chromosome inactivation (XCI) is the transcriptional silencing of one of the two X chromosomes in female mammalians. Females are functional mosaics for two cell lines, one with the maternal X and one with the paternal X as the active chromosome. This mosaicism has important implications for the phenotypic expression of X-linked diseases (M. F. Lyon, 2002). Although XCI analysis is widely used, indications and limitations for the use in clinical practice have received little attention.

Sex chromosomes differ significantly in their gene content (Adler et al., 1997), which has led to the evolution of mechanisms of dosage compensation (LYON, 1962; Mary F. Lyon, 1961). At least three different mechanisms have been described to adjust sex chromosome gene expression dosage between males and females. The single male X-chromosome is hyper transcribed in flies; both X-chromosomes are partially repressed in worms, while in mammals one of the two X-chromosomes is silenced in each cell (Mary F. Lyon, 1961). It was 50 years ago that cytological observations in mouse, rat, opossum and human (LYON, 1962; Ohno & Hauschka, 1960) resulted in the Lyon hypothesis (1961), whereby dosage of X-chromosomal gene products is equalized between male and female mammals, by inactivating one of the X-chromosomes during early development in females (LYON, 1962; Mary F. Lyon, 1961). Fifty

years on, we are still seeking the exact mechanisms that trigger the initial differential treatment of the two X-chromosomes, as well as the spread and maintenance of the inactive state. The aim of this review will be to describe the mechanisms of X-chromosome inactivation in different animals.

In mammals, dosage compensation for X-linked gene products between the sexes is achieved by X-chromosome inactivation (XCI) in females (Mary F. Lyon, 1961). This process leads to the highly regulated transcriptional silencing of one of the two X-chromosome during early development, leading to the formation of the heterochromatic Barr body. X inactivation is an outstanding example of chromosome wide epigenetic regulation involving the developmental silencing of approximately one thousand genes. XCI shares many of the features of others epigenetic mechanisms such as a mosaic cellular phenotype; mitotic heritability but developmental reversibility of X-chromosome inactivity; asynchronous DNA replication timing compared to the rest of the genome; and finally a combination of several epigenetic mechanisms including DNA promoter methylation, histones post-translational modifications, and an unusual nuclear organization. These various features are believed to act synergistically to maintain the X inactive state. Thus, the inactive X chromosome represents a remarkable illustration of the numerous epigenetic mechanisms that can underlie the formation and maintenance of facultative heterochromatin throughout the lifetime of mammals. Pathologists have long noted that the heterochromatic structure of the Barr body was frequently absent in breast cancer cells, particularly in the most aggressive tumors (BARR & MOORE, 1957; Perry, 1972). This observation was later found to be due to the frequent genetic loss of the Xi, with reduplication of the Xa also occurring in some cases in cancer cells resulting in a double dose of X-linked genes. However, another mechanism for Barr body loss in cancer that has been proposed involves the decompaction of its heterochromatic structure, which may be accompanied by X-linked gene reactivation.

LITERATURE REVIEW

X -chromosome inactivation and their history

Inactivation of one of the two X chromosomes occurs in early embryonic life, about the time of implantation, and is random and permanent for all descendants of a cell. However, not all of the more than one thousand genes on the X chromosome are inactivated. Genes that escape X inactivation are spread throughout the X chromosome, although the majority are located on the short arm, especially in the pseudoautosomal region. Escape from X inactivation may be incomplete, and may vary between tissues and between individuals. Extensive investigation of the expression of X-linked genes showed that 15% escaped X inactivation to some degree. For an additional 10%, X inactivation varied between females. This variation in gene expression between females may explain a portion of the phenotypic variation in females in X-linked disorders (Ørstavik, 2009).

The story began in 1953 when the first mouse X-linked genes, tabby and mottled, were discovered, and the heterozygous females showed variegation. In 1959, chromosomally XO mice were shown to be viable fertile females, indicating that only a single

X-chromosome was needed for normal female development in the mouse. At around the same time, the sex chromatin body, discovered ten years earlier by Barr & Bertram, was shown by Ohno to consist of one condensed X-chromosome. It was at this point that the discovery of another X-linked gene giving variegated heterozygotes triggered the formulation of the hypothesis that one X-chromosome was inactivated in the somatic cells of mammals (Mary F. Lyon, 1961). A very similar idea was put forward by (Beutler et al., 1962) to account for the presence of two types of red blood cells in human females heterozygous for X-linked deficiency of glucose-6-phosphate dehydrogenase, and (Russell, 1961) suggested a similar, but rather less complete, explanation for variegation in female mice carrying X autosome translocations.

After the discovery of the phenomenon, then there followed a rapid period of setting out the details. Within a short time the hypothesis was modified to say not that one X-chromosome was inactivated, but rather that a single X-chromosome remained active, in view of the number of sex chromatin bodies seen in individuals with abnormal numbers of sex chromosomes (e.g. XXX, XXY, and XXXX). A problem for the theory was that these individuals showed phenotypic abnormalities, which were particularly severe in human XO females. To take account of this, it was suggested early on that there could be a non-inactivated region on the X-chromosome that corresponded with a homologous region on the Y and so would not require dosage compensation (LYON, 1962). Another early development of the theory concerned germ cells. Ohno found that both X-chromosomes appeared noncondensed and presumably, therefore, active in female germ cells. A little later the single X-chromosome in male germ cells was shown to be inactive. Thus, single X-chromosome activity was a feature of somatic cells only (M. F. Lyon, 1970). Germ cells were postulated to need differing X-chromosome dosage in the two sexes, with sterility resulting from germ cell death in sex chromosome aneuploids.

An early concept concerning the mechanism of X-inactivation was that of an X-inactivation center on the X-chromosome from which inactivation was postulated to spread (Russell, 1961). This idea was suggested to take account of the effects seen in female mice with X-autosome translocations. Inactivation appeared to spread from the X into attached autosomal material, but only into one of the two segments of the translocation. Furthermore, the spread of inactivation into autosomal material appeared limited in comparison with that in the X itself. To understand the nature of the spreading signal it was appreciated that it would be necessary to find the causal relations among the properties of the inactive X: its condensation, late replication, and lack of transcription (Shevchenko et al., 2013).

Thus, ideas on X-inactivation, broadly in line with those accepted today, were already in place in the 1960s, and it may be asked why progress was not more rapid. To answer this question, one must remember how little was known of mammalian genetics, whether of human, mouse, or any other species, at that stage. Suitable genetic variation for study was not available. A milestone came with the formulation of what is now known as Ohno's Law. Ohno put forward the idea that, because of the different dosage relationships of autosomal and X-linked genes, translocations between the X and autosomes that occurred during evolution would be detrimental and would be eliminated. Hence, genes X-linked in one mammalian species would

be X-linked in all. This hypothesis opened up a way to find X-linked genes for study of X-inactivation in any species and hence enabled advances in the field. Ohno's Law is now very well established with no exceptions so far known among eutherian mammals. However, there have been interesting findings recently concerning marsupials and monotremes. Genes on the long arm (Xq) of the present-day human X-chromosome are again X-linked in these groups, but genes on the human short arm (Xp) are autosomal both in marsupials and in the monotreme, the platypus, suggesting that these genes have been recruited from autosomes to the X-chromosome during the evolution of eutherian mammals. Genes on the human and mouse X-chromosomes have been rearranged relative to each other in evolution and genes from Xp are found in at least three separate segments of the mouse X-chromosome (M. F. Lyon, 1988). Presumably, the arrangement on the human X is nearer to that on the primitive eutherian X-chromosome.

Dosage compensation

X chromosome inactivation is believed to occur in order to achieve dosage equivalence between mammalian females who have two X chromosomes and males, who have a single X chromosome and the sex determining Y chromosome. In other organisms with sex chromosomes there is a similar need for dosage compensation; however, the mechanism of achieving equivalent sex chromosomal gene expression is remarkably varied. For example, in *Drosophila*, genes on the single X in XY males are hyper-transcribed, while in *Caenorhabditis*, genes on both X chromosomes in XX hermaphrodites are down-regulated. Despite the very different outcomes, these processes all involve chromatin modifications and both the *Drosophila* and mammalian processes involve a functional RNA.

Since the need for dosage compensation arises from the divergence of the X and Y chromosomes, it was anticipated that genes shared between the two chromosomes, particularly those in the pairing regions of the X and Y (the pseudoautosomal regions - PAR), would not be subject to X inactivation (LYON, 1962). This is indeed the case, and these genes are said to escape inactivation. Surprisingly, there are also a large number of additional genes on the human X chromosome that escape inactivation (Carrel et al., 1999; Carrel & Willard, 2005).

Genes that Escape X Inactivation

Females are normally mosaic for populations of cells with each X active, complicating the determination of whether a gene is subject to inactivation. It was not until 1979 that definitive evidence was provided that a gene, the human steroid sulphatase (*STS*) locus, could escape inactivation. Individual clones of fibroblasts were isolated from female carriers for X-linked icthyosis due to *STS* deficiency, who were also heterozygous for *G6PD.* Clones expressing either allele of *G6PD* continued to express *STS*, demonstrating that even when the *STS* mutation was on the active X, the inactive X must have been contributing STS (Mohandas et al., 1980). To assess inactivation status of genes without the need for clonal populations and polymorphisms or mutations to distinguish the two X chromosomes, many studies have utilized somatic cell hybrids, which can be selected to retain either the active or inactive human X chromosome (Willard et al., 1993). Using a panel of hybrids retaining the inactive X, Carrel *et al.* have surveyed over 600 X-linked transcripts, and demonstrated that a surprising 15% are

expressed from the inactive X (Carrel & Willard, 2005). These genes show a non-random distribution, with the majority of 'escapees' found on the short arm of the X, which is an evolutionarily more recent addition to the eutherian X being autosomal in marsupials and monotremes (Watson et al., 1992). Surveying inactivation of three X linked genes across many eutherians, Jegalian and Page hypothesized that it is the deterioration of the Y homologue that drives inactivation of the X-linked copy (Jegalian & Page, 1998). Thus it may be that genes on the short arm have lost their functional Y copy more recently and have not yet acquired complete inactivation.

Differences between Humans and Mice

Lyon first hypothesized inactivation based on her studies of mice, and since then the mouse has been a powerful model system for the study of X inactivation. Murine female embryonic stem (ES) cells undergo inactivation upon *in vitro* differentiation. Manipulation of these cells by transgene insertion and homologous gene targeting, combined with the ability to reconstitute mice allowing *in vivo* studies, have made mouse ES cells a highly tractable model system for the study of inactivation. However, there are several known differences between inactivation in humans and mice, two of which are particularly relevant. First, it appears that more genes escape inactivation in humans. Although not as many genes have been examined in mouse, the idea that more human genes are expressed from the inactive X is consistent with the development of Turner syndrome in human females lacking a sex chromosome in contrast to the apparently normal phenotype of such mice. Second, imprinting of inactivation varies amongst species. In marsupials the paternal X chromosome is preferentially inactivated in all tissues (Cooper et al., 1971), while in mice preferential paternal inactivation is observed only in the extraembryonic tissues (Takagi & Sasaki, 1975). In humans there have been reports of non-random inactivation in extraembryonic tissues; however it is clear that in general this is not a stringent imprint (*e.g)* (Looijenga et al., 1999).. The extraembryonic tissues are the first tissues to undergo inactivation in mice, and the imprint is lost by the time the somatic tissues undergo inactivation (Mak et al., 2004; Ikuhiro Okamoto et al., 2004). Thus the absence of imprinting in humans may reflect a delay in timing of inactivation until imprint loss has already occurred, however it might also reflect differences in the initial events of inactivation, which are currently only well-studied in the mouse model.

PHENOMENOLOGY OF X-INACTIVATION IN MAMMALS

Monotremes use a mechanism different from X chromosome inactivation for dosage compensation in the living representatives of the most ancient mammalian subclass Prototheria, one platypus and four echidna species, are merged into the order of monotremes (Monotremata). Unlike the rest of mammals, the monotremes have a complex sex-determination system. The male platypus (*Ornithorhychus anatinus*) has five X and five Y chromosomes; five X and four Y chromosomes have been detected in male echidna (*Tachuglossus aculeatus*). The genes typical of the X chromosomes of marsupial and eutherian mammals have autosomal localization. However, the genes typical of the sex chromosome Z of birds (including the *Dmrt1* gene, which presumably plays the key role in sex determination in birds) have been found on the X chromosomes of monotremes. The most extensive region

homologous to the chicken Z chromosome has been detected on the platypus X5 chromosome; less extensive regions of homology are localized on the X_1, X_2, and X_3 chromosomes (*Fig. 1*).

Chromosome	Gene	Ratio between the gene expression levels in females and males	Fraction of nuclei with monoallelic expression
Complete compensation			
X_1	*Ox_plat_124086*	1.10	46
X_2	*ZNF474*	1.01	53
X_3	*LOX*	1.06	53
X_4	*APC*	1.17	48
X_5	*SHB*	1.23	53
Partial compensation			
X_1	*FBXO10*	1.37	50
X_3	*EN14997*	1.40	61
No compensation			
X_4	*SEMA6A*	1.82	74
X_3	*DMRT2*	2.04	47
X_5	*SLC1A1*	2.78	45

Table1. The ratio between the gene expression levels in the X chromosomes in female and male platypus cells and frequency of their monoallelic expression.

All the X and Y chromosomes of monotremes contain homologous pseudoautosomal regions that enable conjugation between the X and Y chromosomes in meiosis. However, the extensive regions of the platypus X_1–X_5 chromosomes (corresponding to ~12% of the genome) are non-homologous and show no similarity to Y_1–Y_5. It is reasonable to expect that a mechanism of dosage compensation for the genes localized in these regions exists. A quantitative analysis of the transcription of the genes localized in the differentiated regions of different platypus X chromosomes has demonstrated that some of them have identical transcription levels both in female and male cells, while expression of the remaining genes is either compensated partially or is not compensated at all (i.e., expression in female cells turns out to be twice as high as that in male cells) (*Table 1*). Thus, dosage compensation in monotremes presumably functions only for individual genes of the sex chromosome, resembling incomplete and variable dosage compensation in birds. In cell nuclei of female platypus, transcription of the genes exhibiting dosage compensation is revealed only for one of the homologous X chromosomes with a frequency of 50–70%. Nevertheless, total mRNA contains equal amounts of transcripts corresponding to each homologue. These data provide grounds for assuming that dosage compensation in monotremes occurs due to a decrease in the transcription level of one of the alleles (selected in each cell in a random manner). Since each pair of X chromosomes in female platypus has no visible distinctions in chromatin modifications at the cytological level, it is assumed that the dosage compensation in monotremes affects individual genes rather than chromosomes (Shevchenko et al., 2013).

The pseudoautosomal region of the echidna X1 chromosome in some cell types is characterized by late replication, which can be regarded as an indicator of inactive chromatin, although the genes localized in this region are present both on X_1 and Y_1 and require no dosage compensation. Taking into account its susceptibility to inactivation, this region was previously regarded as an ancestral region when the mechanism of silencing of an entire chromosome could have presumably been formed. However, since the genes contained in this region in marsupial and eutherian mammals have autosomal localization and are not involved in inactivation, this assumption has been refuted (Shevchenko et al., 2013). Thus, it is an obvious fact that monotremes, unlike marsupial and eutherian mammals, use a mechanism that differs from X chromosome inactivation for dosage compensation.

Imprinted, incomplete and tissue-specific X chromosome inactivation in marsupial mammals

Infraclass Metatheria (marsupials) comprises 270 species, 200 of which live in Australia; 69, in South America; and 1, in North America. The evolutionary segregation between Australian and American marsupials occurred 70 million years ago. The sex chromosomes in marsupial and eutherian mammals have a common origin. The X chromosome in marsupials represents 2/3 of the X chromosome of eutherian mammals; the remaining third of the genes are localized on the autosome (*Fig. 1*). Marsupials are the most ancient mammals; dosage compensation in female marsupials occurs due to X chromosome inactivation; however, the inactivation processes in marsupial and eutherian mammals differ significantly (Shevchenko et al., 2013).

Nonrandom imprinted inactivation is typical of all marsupial tissues; it involves suppression of gene transcription and establishment of late replication in the S phase of the cell cycle, exclusively on the X chromosome inherited from the father. The untranslated nuclear rnRNA *Rsx* (RNA-on-the-silent X), which can propagate over the inactive X chromosome and repress gene transcription, is presumably responsible for the inactivation process at the chromosomal level. The imprinted inactivation of three genes of the X chromosome has been studied in tissues of eight species (*Table 2*). It was found that the inactive status of the X chromosome inherited from the father is unstable, and that genes are frequently reactivated. It turns out that inactivation in marsupials does not affect all genes to the same extent (i.e., is incomplete). Moreover, the same loci of the X chromosome can be inactivated to different extents depending on a particular tissue. Thus, the phosphoglycerate kinase A (*Pgk1*) gene in the Virginia (North American) opossum *Didelphis virginiana* is completely inactivated in all tissues, whereas no stable repression of the paternal allele of the glucoso-6-phosphate dehydrogenase (*G6pd*) gene is observed in most tissues. In the gray short-tailed opossum *Monodelphis domestica*, unlike the Virginia opossum, the paternal *G6pd* allele is stably inactivated, whereas*Pgk1* exhibits incomplete inactivation in all tissues. Thus, orthological genes can be inactivated to different extents in different marsupial species (Shevchenko et al., 2013).

Gene	Species	Method	Inactivation in somatic tissues
G6pd	*Macropus robustus*	Isoenzyme analysis, SNuPE	Complete
	Macropus rufogriseus	Isoenzyme analysis	Complete
	Didelphis virginiana	Isoenzyme analysis	Partial
	Monodelphis domestica	RT-PCR	Complete
Gla	*Antechinus stuarttii*	Isoenzyme analysis	Complete
	Kangaroo hybrids	«	Complete
Pgk1	*Macropus giganteus*	«	Tissue-specific
	Macropus parryi	«	«
	Trichosurus vulpecula	«	«
	Didelphis virginiana	«	«
	Monodelphis domestica	SNuPE	Partial

Table2. Status of gene expression in the X chromosomes in different marsupial species

It should be mentioned that X chromosome inactivation is not the only mechanism of dosage compensation in marsupials. In the members of the bandicoot family (Paramelidae), the Y chromosome in males and one of the two X chromosomes in females are eliminated at different ontogenic stages in somatic cells. The elimination of sex chromosomes in different tissues can be observed either in all cells or in some of them. The investigation of the expression of the alleles of the X-linked *Pgk1* gene in the southern brown bandicoot *Isoodon obesulus* shows that only the X chromosome inherited from the father is eliminated in females. In the cells where sex chromosomes have not been eliminated, the X chromosome of paternal origin in females and the Y chromosome in males are late-replicating. The mechanism of elimination of sex chromosome is unknown; however, the preferential elimination of the X chromosome inherited from the father and asynchronous replication of the X chromosomes in females attest to the fact that this process emerged in marsupials as a trend in the evolution of the X chromosome inactivation process (Shevchenko et al., 2013).

Eutherian mammals

Infraclass eutheria (placental mammals), which is subdivided into the four superorders Afrotheria, Xenarthra, euarchontoglires and Laurasiatheria, the most numerous, diverse, and common mammalian infraclass. The X chromosome in eutherian mammals consists of the genes constituting the X chromosome in marsupials by 2/3 and contains an added region, which has autosomal localization in marsupials (Fig. 1). As opposed to marsupial mammals, the X chromosomes of paternal and maternal origins are inactivated with equal probabilities in the cells of adult female eutherians; hence, on average half of the cells express the genes of the paternal X chromosome, while the remaining half express the genes of the maternal X chromosome. Unlike imprinted inactivation, random inactivation embraces most genes on the chromosome and is stably maintained through cell generations. It should be mentioned that the genes in the added region of the X chromosome in eutherian mammals, which were localized on the autosome in marsupials and did not participate in the inactivation process, are inactivated

with a lower efficiency and are capable of avoiding inactivation. The random inactivation in eutherians comprises several stages: counting the number of X chromosomes per diploid genome, choice of an X chromosome for inactivation, initiation of activation, and propagation of the inactive status and its maintenance through cell generations. It is possible that the stage involving the choice of the X chromosomes (during which the mutually exclusive choices of the future active and inactive X chromosomes (like in a mouse) occurs) is typical not of all eutherian species. thus, inactivation in early ontogenesis of the rabbit occurs stochastically, resulting in the formation of different cells, where 1) neither one of the X chromosomes is inactivated, 2) both X chromosomes are inactivated, or 3) one X chromosome out of two is randomly inactivated. Due to the disrupted gene dosage, the former two cell types subsequently die, while the remaining cells with normal inactivation form the organs and tissues of the organism (Shevchenko et al., 2013).

In certain taxa of eutherian mammals (e.g., in rodents and artiodactyles), in addition to the random inactivation there also exists imprinted, incomplete and unstable inactivation of the X chromosome inherited from the father (however, this occurs exclusively at the pre-implantation stages of embryogenesis and remains in cells resulting in extra embryonic organs (placenta and vitelline sac) (Shevchenko et al., 2013).

Both the random and imprinted inactivation in eutherians are controlled by the inactivation center (XIc) and the *Xist* gene, which have not been detected in monotremes and marsupials. During the random inactivation, the *Xist* gene ensures initiation of inactivation and propagation of the inactive status, while the other elements of the inactivation center function at the stage of the counting of X chromosomes and choice of the chromosome to undergo inactivation (Shevchenko et al., 2013).

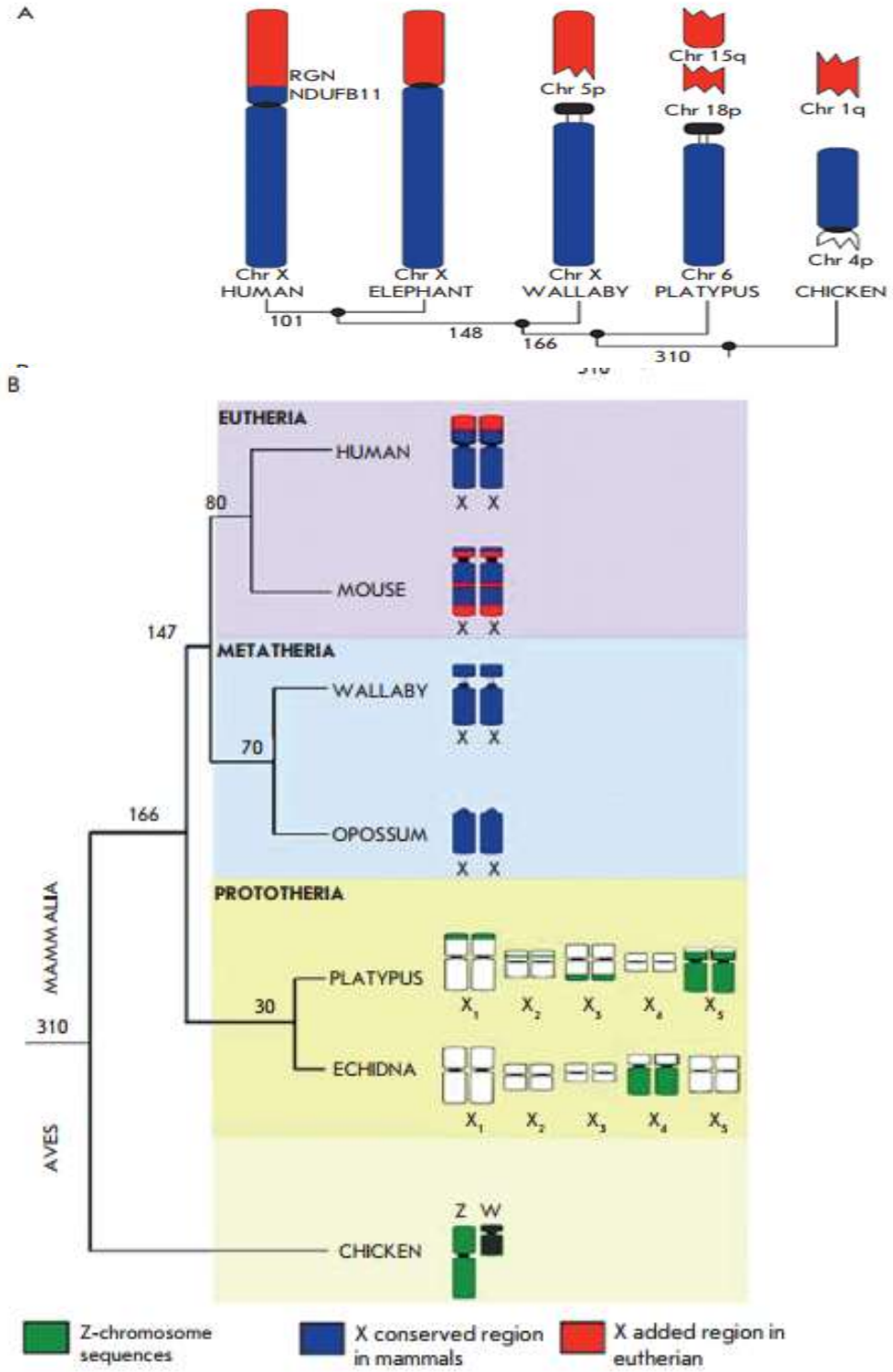

Fig1. The origin and evolution of the mammalian X chromosome. A) Genes of the mammalian X chromosome have autosomal localization in birds (chicken) and monotremes (platypus, echidna). The X chromosome of marsupials (wallaby, opossum) represents the most ancient

part of the mammalian X (shown in blue) and comprises 2/3 of the genes of the eutherian X chromosome. The eutherian X chromosome contains an added region (shown in red), which has autosomal localization in marsupials [7]. B) Monotremes have five X chromosomes, which show nothing in common with eutherian X but contain sequences homologous to the Z chromosome of birds [9]. The divergence time of the taxa (Mya) is shown on the branches of the phylogenetic tree

X-chromosome inactivation in methatherian species

The metatherian and eutherian lineages segregated 148 million years ago, following the emergence of Sry. Marsupial XCI is characterized by the inactivation of the paternally inherited X-chromosome in embryonic and extra-embryonic tissues (Dupont & Gribnau, 2013). Methatherian XCI is established, similarly to the series of events in eutherians, via a vast array of chromatin modifications. However, XCI seems to be less stable and more tissue-specific in marsupialsin comparison to placental. A genome wide comparison of the region surrounding Xist has indicated that Xist evolved after the divergence of the eutherian and metatherian lineages (Duret et al., 2006). Recent work has indicated that another Xi associated non-coding RNA, transcribed from the X-linked Rsx gene, and has evolved in metatherian metatherians. Similar to Xist, Rsx is a large RNA (>17 kb) that coats the Xi, and can initiate silencing when ectopically expressed from autosomes in eutherian cells. Consequently, it is thought that dosage compensation evolved independently in metatherians and eutherians, to oppose up regulation of X-linked genes for which the Y-homologous genes (gametologs) were lost from the Y chromosome. This also implies that massive degeneration of the Y chromosome only started after the split of the eutherian and metatherian lineages.

Early embryogenesis differs vastly between placental and marsupial species. In contrast to eutherians, marsupial blastocysts are initially unilaminar and do not display discernible differences between cells. Interestingly, the pluripotency gene REX1 is absent in marsupials, and originated in eutherians through retrotransposition. If Rsx and Xist mediated iXCI was the ancient form of XCI in metatherians and eutherians, respectively, the later acquisition of REX1 in eutherians may have directed ICM development, and may have provided a mechanistic opportunity for X chromosome reactivation and subsequent rXCI in eutherian species.

Imprinted X-chromosome inactivation (XCI)

XCI in mice is dependent on the well-controlled and interrelated expression of two non-coding RNAs, Xist (Borsani et al., 1991; Brockdorff et al., 1991) and its antisense transcript Tsix. Whereas Xist is transcribed from the X chromosome that undergoes XCI, Tsix is transiently expressed from the transcriptionally active X chromosome before, and during initiation of XCI. Xist accumulation on the X chromosome is followed by recruitment of remodeling complexes which have been implicated in transcriptional silencing of the X chromosome. Gene expression from the X chromosomes is bi-allelic immediately following zygotic genome activation (Namekawa et al., 2010; Patrat et al., 2009). Soon afterwards, Xist accumulates exclusively on the Xp, followed by a progressive initiation of XCI from the 4-cell stage onwards (Patrat et al., 2009). This iXCI results in the exclusive inactivation of the Xp. IXCI is maintained in lineages arising from the primitive endoderm (PE) and the trophectoderm (TE), but will be modified in

lineages emerging from the epiblast (Nesterova et al., 2001; I. Okamoto et al., 2000; Takagi & Sasaki, 1975). Xist transcripts on the Xp are lost in nascent epiblast cells of blastocysts, but subsequently reemerge on day E5.5 to E6.5 in a non-imprinted manner, either on Xp or Xm (Mak et al., 2004; Ikuhiro Okamoto et al., 2004). Whereas Xist and Tsix deletion studies have advocated that a maternal imprint prevents Xist transcription from the Xm during the early cleavage stages (Marahrens et al., 1997; Sado et al., 2001), nuclear transfer experiments provided evidence that the maternal imprint on the X chromosomes from oocytes is acquired during follicular growth (Tada et al., 2000). Hypothetically, a maternal imprint could be located either on Tsix maintaining its transcription or on Xist preventing its expression. The failure to detect Tsix before the blastocyst stage, however, has fueled the argument that the maternal imprint has a direct impact on Xist transcription rather than on Tsix transcription (Sado et al., 2001). The nature of the imprint is unknown. OCT4 and SOX2 have been proposed to act as allele specific repressors of Xist during early development following their binding to the Xist intron 1 region of the Xm. The binding of OCT4 and SOX2 to DNA occurs in a co-operative manner, which therefore necessitates the simultaneous presence of both transcription factors (D C Ambrosetti et al., 1997; Davide Carlo Ambrosetti et al., 2000). SOX2, however, is absent in blastomere nuclei before the morula stage (C.D. unpublished observation) and the functionality of the OCT4-SOX2 hetero-dimer is repressed by CDX2 at a later stage (Niwa et al., 2005). Therefore, it is unlikely that OCT4 and SOX2 act as allele specific repressors of Xist during early cleavage stages. A crucial factor for rXCI is the ubiquitin ligase RNF12 (Barakat et al., 2011), which targets the Xist-repressor REX1 for degradation. RNF12 is highly expressed in the cleavage stage embryo and is also crucial for iXCI, where it may induce Xist expression from the Xp or degrade a Xistrepressor, possibly REX1. It seems therefore that iXCI is maintained by a strong maternal imprint preventing Xist expression from the Xm, acting in combination with factors that directly or indirectly induce Xist expression from the Xp.

The process of X- inactivation

The X Inactivation Center (XIC)

In diploid mammals all X chromosomes in excess of one are inactivated, suggesting an initial marking of a single active X. Early studies of X chromosome rearrangements identified a region required in *cis* for inactivation of the chromosome, and demonstrated that inactivation can spread from this region into translocated autosomal material (Russell, 1963). Studies of human X chromosome rearrangements refined the *XIC* to an approximately one Mb region of Xq13 (Lepplg et al., 1993), while mouse transgene studies have identified a 450 kb region that can recapitulate the features of the *Xic* (Lee et al., 1996) (standard nomenclature dictates that the lower case naming is for the mouse locus, while the upper case name is the designation for the human). The *Xic* region (see Figure 2) contains seven protein-encoding genes, none of which have been shown to be involved in the X-inactivation process. All the genes identified in this region in mouse are conserved in human, except *Ppnx* and *Tsix* (which has become a pseudogene in human). The region encodes a number of non-coding RNAs (see Figure 2) and also has a high density of pseudogenes (22 in 714 kb), which may in part be related to the transcriptionally active nature of this genomic region in the testis, as three (*Tsix*, *Ppnx*, and

Cnbp2) of eleven genes in the region are specifically expressed in the testis (Chureau et al., 2002). The most striking of the genes in the region is the X-Inactive Specific Transcript gene (*Xist*/*XIST),* which is required for inactivation. The region includes elements that are required for the marking of an active X chromosome and the stable expression and localization of *XIST* from the inactive X chromosome.

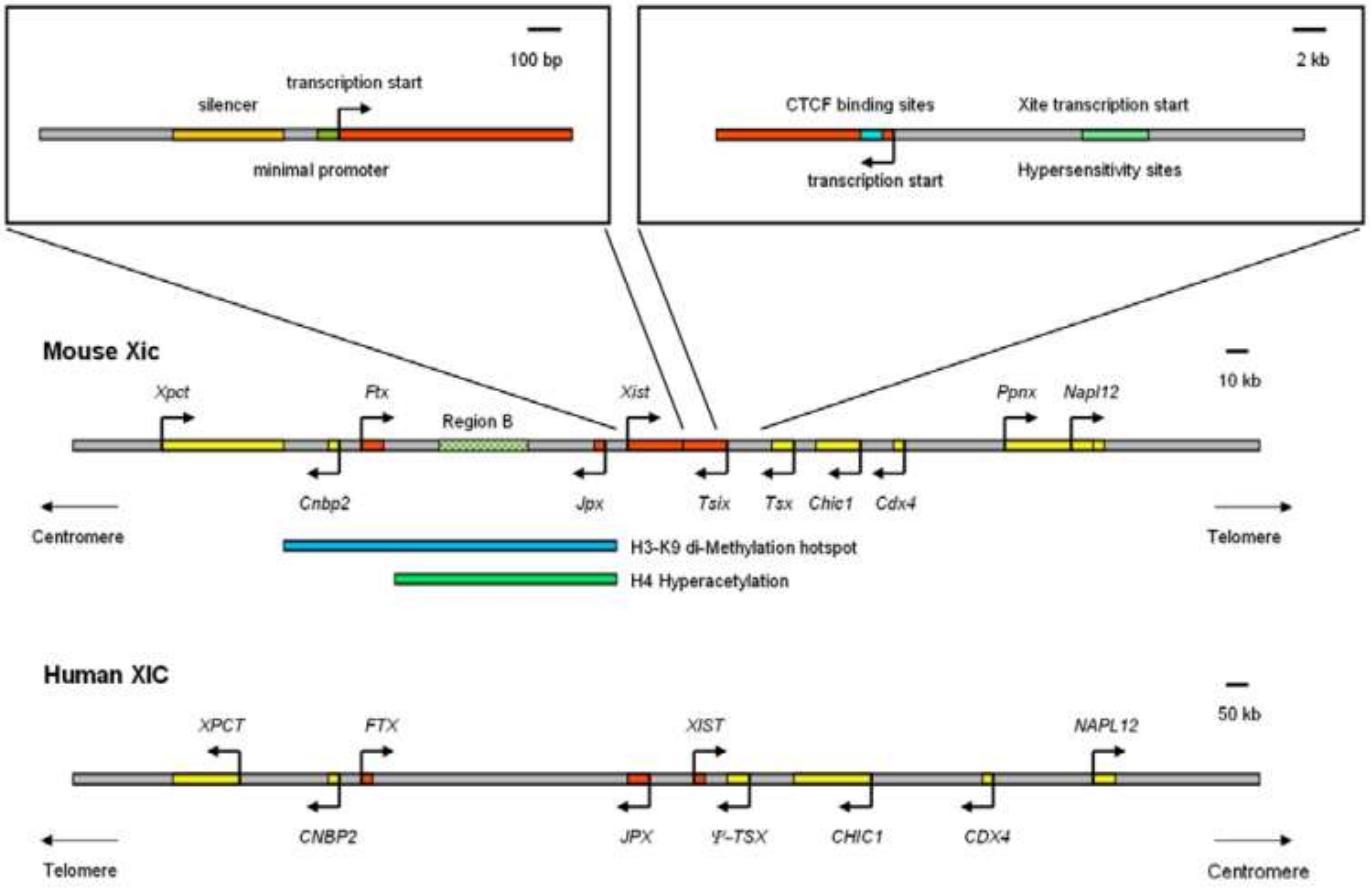

Figure2. Transcription maps of the *Xic*/*XIC* regions in mouse and human (Chureau et al., 2002). There are 11 genes in the mouse *Xic* region: *Xpct, Xist, Tsx, Tsix, Chic1* (formerly, *Brx*), *Cdx4*, *NapIl2* (formerly, *Bpx*), *Cnbp2, Ftx, Jpx*, and *Ppnx*. Protein coding genes are represented by yellow boxes. Four of the 11 genes, *Xist, Tsix, Ftx*, and *Jpx,* are untranslated RNA genes and represented by red boxes. Region B, a non-coding expressed domain, is represented by a striped box. All the genes identified in mouse are conserved in human, except *Ppnx* and *Tsix*. In human, however, *Tsx* has become a pseudogene. The human region is approximately three times larger than the mouse. Despite this major change in size, the order and orientation of genes is conserved in human and mouse, except for *Xpct*, which is at the same location but in the inverse orientation. A histone H3 lysine 9 dimethylation hotspot and H4 hyperacetylation are represented by blue and green boxes below the transcription map of the *Xic* region in mouse. Pillet *et al.* showed that the region -1157 to +917 has no *in vitro* sex-specific promoter activity (Pillet et al., 1995). A minimal constitutional promoter was assigned to a region from -81 to +1. Deletion of the segment -441 to -231 is associated with an increase in CAT activity and may represent a silencer element. The choice/imprinting center contains tandem CTCF binding

sites. Chao *et al.* proposed that *Tsix* and CTCF together establish a regulatable epigenetic switch for X-inactivation (Chao et al., 2002). Ogawa and Lee showed that *Xite*, located 10 kb from the *Tsix* transcription start, harbours two clusters of DNase hypersensitive sites (Ogawa & Lee, 2003).

The X-chromosome inactive specific transcript (XIST) Gene

Xist/*XIST* is a large (>15 kb) alternatively processed, poly-adenylated, untranslated RNA that is the only gene known to be transcribed from the inactive but not from the active X chromosome in somatic cells. The gene lacks any conserved open reading frame, and presumably functions as RNA. In interphase nuclei, the colocalization of the Xist/XIST RNA with the inactive X territory as part of the heterochromatic Barr body is suggestive of an involvement in X inactivation. A direct requirement for *Xist* in X inactivation was demonstrated by 'knock out' of *Xist* in female ES cells which abolished X inactivation potential in *cis*. Furthermore, transgenes of *Xist*, integrated as multi-copy arrays, are able to induce inactivation of autosomes, identifying *Xist* as the principle component of the *Xic* (Rougeulle & Avner, 2003). Prior to inactivation, *Xist* expression is detected as a small pinpoint of expression from both X chromosomes, until the transcripts accumulate and localize on the future inactive X, mediated at least in part by stabilization of the transcript (Panning et al., 1997; Steven A. Sheardown et al., 1997). While localization and stability are developmentally concurrent, they have been experimentally separated, with stable transcripts failing to localize (Chow et al., 2003; Clemson et al., 1998; Hansen et al., 1998; Wutz et al., 2002). The puzzle of how one of two apparently equivalent X chromosomes can be chosen to express *Xist*, and thus be inactivated, remains to be solved. It is clear, however, that components of the *Xic* are involved, and it has been suggested that the levels of Xist RNA may influence which X undergoes inactivation (Nesterova et al., 2003).

Xist Gene Regulation

The *Xist*/*XIST* promoter regions in mouse and human (see Figure 2) are constitutively active, containing binding sites for ubiquitous transcription factors and lacking sex-specific activity (Hendrich et al., 1997; Pillet et al., 1995; S. A. Sheardown et al., 1997). Thus, additional elements must be responsible for the silencing of *XIST* in males and expression from a single X in females. DNA methylation has been implicated in the regulation of *Xist*/*XIST* in differentiated cells since the promoter region of the transcriptionally active allele on the inactive X chromosome is unmethylated, whereas that of the transcriptionally inactive allele on the active X chromosome is methylated (Hendrich et al., 1993; Norris et al., 1994). Loss of *XIST/Xist* methylation results in expression of the gene in somatic (Beard et al., 1995; Hansen et al., 1998; Tinker & Brown, 1998), but not embryonic cells (Beard et al., 1995), suggesting that methylation is a late event in the control of *XIST* expression. Elements both 5' and 3' of the *Xist* gene appear to regulate the function of *Xist* early in development, and are thus important components of the *Xic*.

Factors Interacting with Xist RNA

Expression of *Xist* is the initiating step in X inactivation, but it remains to be determined how the RNA localizes to the chromosome from which it is transcribed, and how localized *Xist*

initiates the cascade of heterochromatinzing events that lead to a stably silent inactive X chromosome. Analysis of partial deletions of the Xist RNA have shown separable domains, with the capacity to associate with the inactive X chromosome involving three spatially distinct and apparently redundant regions of the *Xist* RNA, while deletion of the conserved 5' repeat eliminates the ability to silence without disrupting the ability to localize (Wutz et al., 2002). These repeats show sequence conservation in all species in which *Xist* has been analyzed and are predicted to form an RNA hairpin (Hendrich et al., 1993; Wutz et al., 2002). Only RNAs that are capable of coating the chromosome are able to silence (Wutz et al., 2002). While no specific interactions with the various domains of the Xist RNA, and proteins have been identified that associate with the inactive X, and thus might interact with XIST/Xist. These include the variant histone macroH2A, the Eed/Ezh2 polycomb complex that modifies histones, and *BRCA1,* which has been reported to be necessary for *XIST* localization (Ganesan et al., 2002). Many mammalian homologues of the PRC1 protein complex that maintains homeotic gene silencing in *Drosophila* have also been shown to associate with the inactive X in human and/or mouse somatic cells (Plath et al., 2004).

CONCLUSION

The inactive X chromosome provides a remarkable example of chromosome-wide gene repression that reveals the diversity of processes that can contribute to the formation of facultative heterochromatin. XCI also highlights the complex issue of gene dosage. The repeated inactivation and reactivation of the X chromosome during development illustrates the importance of a fine regulation of all the mechanisms involved. Recent studies have revealed that a combination of chromatin factors, chromosome conformation and nuclear compartmentalization together ensure maintenance of the inactive state. The mechanism that triggers gene silencing in the first place, via Xist RNA remains tantalizingly obscure. Once XCI is established, silencing appears to be rather stable in terminally differentiated cells, although XCI is less complete in humans than in mice and some genes can escape this silencing process, either for a purpose or accidentally.

Thus, it can be said that the process of X chromosome inactivation in marsupial and eutherian mammals has common epigenetic and, possibly, molecular mechanisms. The key feature of the inactivation process in mammals, the coordinated gene repression at the level of the X chromosome, is presumably a result of the propagation of the nuclear noncoding RNA along it. However, the *Rsx* gene of nuclear noncoding RNA was replaced in eutherians during evolution by *Xist*, which is better, as compared to its ancestor, at attracting modifications, providing stable gene inactivation, to the X chromosome. The inactivation center with elements capable of counting and choosing the future active and inactive chromosomes was formed around the *Xist* gene, which made random repression of one of the two X chromosome possible. Furthermore, the formation of the more complete and stable inactivation in eutherians was promoted by the involvement of the mechanisms of DNA methylation in the maintenance of the inactive status and enrichment of the X chromosome in LIne1 sequences, which increase the efficiency of propagation of the inactive state.

REFERENCES

Adler, D. A., Rugarli, E. I., Lingenfelter, P. A., Tsuchiya, K., Poslinski, D., Liggitt, H. D., Chapman, V. M., Elliott, R. W., Ballabio, A., & Disteche, C. M. (1997). Evidence of evolutionary up-regulation of the single active X chromosome in mammals based on Clc4 expression levels in Mus spretus and Mus musculus. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(17). https://doi.org/10.1073/pnas.94.17.9244

Ambrosetti, D C, Basilico, C., & Dailey, L. (1997). Synergistic activation of the fibroblast growth factor 4 enhancer by Sox2 and Oct-3 depends on protein-protein interactions facilitated by a specific spatial arrangement of factor binding sites. *Molecular and Cellular Biology*, *17*(11). https://doi.org/10.1128/mcb.17.11.6321

Ambrosetti, Davide Carlo, Schöler, H. R., Dailey, L., & Basilico, C. (2000). Modulation of the activity of multiple transcriptional activation domains by the DNA binding domains mediates the synergistic action of Sox2 and Oct-3 on the Fibroblast growth factor-4 enhancer. *Journal of Biological Chemistry*, *275*(30). https://doi.org/10.1074/jbc.M000932200

Barakat, T. S., Gunhanlar, N., Pardo, C. G., Achame, E. M., Ghazvini, M., Boers, R., Kenter, A., Rentmeester, E., Grootegoed, J. A., & Gribnau, J. (2011). RNF12 activates Xist and is essential for X chromosome inactivation. *PLoS Genetics*, *7*(1). https://doi.org/10.1371/journal.pgen.1002001

BARR, M. L., & MOORE, K. L. (1957). Chromosomes, sex chromatin, and cancer. *Proceedings. Canadian Cancer Conference*, *2*.

Beard, C., Li, E., & Jaenisch, R. (1995). Loss of methylation activates Xist in somatic but not in embryonic cells. *Genes and Development*, *9*(19). https://doi.org/10.1101/gad.9.19.2325

Beutler, E., Yeh, M., & Fairbanks, V. F. (1962). The normal human female as a mosaic of X-chromosome activity: Studies using the gene for g-6-pd-deficiency as a marker. *Obstetrical and Gynecological Survey*, *17*(5). https://doi.org/10.1097/00006254-196210000-00048

Borsani, G., Tonlorenzi, R., Simmler, M. C., Dandolo, L., Arnaud, D., Capra, V., Grompe, M., Pizzuti, A., Muzny, D., Lawrence, C., Willard, H. F., Avner, P., & Ballabio, A. (1991). Characterization of a murine gene expressed from the inactive X chromosome. *Nature*, *351*(6324). https://doi.org/10.1038/351325a0

Brockdorff, N., Ashworth, A., Kay, G. F., Cooper, P., Smith, S., McCabe, V. M., Norris, D. P., Penny, G. D., Patel, D., & Rastan, S. (1991). Conservation of position and exclusive expression of mouse Xist from the inactive X chromosome. *Nature*, *351*(6324). https://doi.org/10.1038/351329a0

Carrel, L., Cottle, A. A., Goglin, K. C., & Willard, H. F. (1999). A first-generation X-inactivation profile of the human X chromosome. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(25). https://doi.org/10.1073/pnas.96.25.14440

Carrel, L., & Willard, H. F. (2005). X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature*, *434*(7031).

https://doi.org/10.1038/nature03479

Chao, W., Huynh, K. D., Spencer, R. J., Davidow, L. S., & Lee, J. T. (2002). CTCF, a candidate trans-acting factor for X-inactivation choice. *Science*, *295*(5553). https://doi.org/10.1126/science.1065982

Chow, J. C., Hall, L. L., Clemson, C. M., Lawrence, J. B., & Brown, C. J. (2003). Characterization of expression at the human XIST locus in somatic, embryonal carcinoma, and transgenic cell lines. *Genomics*, *82*(3). https://doi.org/10.1016/S0888-7543(03)00170-8

Chureau, C., Prissette, M., Bourdet, A., Barbe, V., Cattolico, L., Jones, L., Eggen, A., Avner, P., & Duret, L. (2002). Comparative sequence analysis of the X-inactivation center region in mouse, human, and bovine. *Genome Research*, *12*(6). https://doi.org/10.1101/gr.152902

Clemson, C. M., Chow, J. C., Brown, C. J., & Lawrence, J. B. (1998). Stabilization and localization of Xist RNA are controlled by separate mechanisms and are not sufficient for X inactivation. *Journal of Cell Biology*, *142*(1). https://doi.org/10.1083/jcb.142.1.13

Cooper, D. W., Vandeberg, J. L., Sharman, G. B., & Poole, W. E. (1971). Phosphoglycerate kinase polymorphism in kangaroos provides further evidence for paternal X inactivation. *Nature New Biology*, *230*(13). https://doi.org/10.1038/newbio230155a0

Dupont, C., & Gribnau, J. (2013). Different flavors of X-chromosome inactivation in mammals. *Current Opinion in Cell Biology*, *25*(3), 314–321. https://doi.org/10.1016/j.ceb.2013.03.001

Duret, L., Chureau, C., Samain, S., Weissanbach, J., & Avner, P. (2006). The Xist RNA gene evolved in eutherians by pseudogenization of a protein-coding gene. *Science*, *312*(5780). https://doi.org/10.1126/science.1126316

Ganesan, S., Silver, D. P., Greenberg, R. A., Avni, D., Drapkin, R., Miron, A., Mok, S. C., Randrianarison, V., Brodie, S., Salstrom, J., Rasmussen, T. P., Klimke, A., Marrese, C., Marahrens, Y., Deng, C. X., Feunteun, J., & Livingston, D. M. (2002). BRCA1 supports XIST RNA concentration on the inactive X chromosome. *Cell*, *111*(3). https://doi.org/10.1016/S0092-8674(02)01052-8

Hansen, R. S., Canfield, T. K., Stanek, A. M., Keitges, E. A., & Gartler, S. M. (1998). Reactivation of XIST in normal fibroblasts and a somatic cell hybrid: Abnormal localization of XIST RNA in hybrid cells. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(9). https://doi.org/10.1073/pnas.95.9.5133

Hendrich, B. D., Brown, C. J., & Willard, H. F. (1993). Evolutionary conservation of possible functional domains of the human and murine Xist genes. *Human Molecular Genetics*, *2*(6). https://doi.org/10.1093/hmg/2.6.663

Hendrich, B. D., Plenge, R. M., & Willard, H. F. (1997). Identification and characterization of the human XIST gene promoter: Implications for models of X chromosome inactivation. *Nucleic Acids Research*, *25*(13). https://doi.org/10.1093/nar/25.13.2661

Jegalian, K., & Page, D. C. (1998). A proposed path by which genes common to mammalian X and Y chromosomes evolve to become X inactivated. *Nature*, *394*(6695). https://doi.org/10.1038/29522

Lee, J. T., Strauss, W. M., Dausman, J. A., & Jaenisch, R. (1996). A 450 kb transgene displays properties of the mammalian X-inactivation center. *Cell*, *86*(1).

https://doi.org/10.1016/S0092-8674(00)80079-3

Lepplg, K. A., Brown, C. J., Bressler, S. L., Gustashaw, K., Pagon, R. A., Willard, H. F., & Disteche, C. M. (1993). Mapping of the distal boundary of the X-inactivation center in a rearranged X chromosome from a female expressing XIST. *Human Molecular Genetics*, *2*(7). https://doi.org/10.1093/hmg/2.7.883

Looijenga, L. H. J., Gillis, A. J. M., Verkerk, A. J. M. H., Van Putten, W. L. J., & Oosterhuis, J. W. (1999). Heterogeneous X inactivation in trophoblastic cells of human full-term female placentas. *American Journal of Human Genetics*, *64*(5). https://doi.org/10.1086/302382

Lyon, M. F. (1970). Genetic activity of sex chromosomes in somatic cells of mammals. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *259*(828). https://doi.org/10.1098/rstb.1970.0044

Lyon, M. F. (1988). The William Allan Memorial Award Address: X-chromosome inactivation and the location and expression of X-linked genes. In *American Journal of Human Genetics* (Vol. 42, Issue 1).

Lyon, M. F. (2002). X-chromosome inactivation and human genetic disease. *Acta Paediatrica, International Journal of Paediatrics, Supplement*, *91*(439). https://doi.org/10.1111/j.1651-2227.2002.tb03120.x

LYON, M. F. (1962). Sex chromatin and gene action in the mammalian X-chromosome. *American Journal of Human Genetics*, *14*.

Lyon, Mary F. (1961). Gene action in the X-chromosome of the mouse (mus musculus L.). *Nature*, *190*(4773). https://doi.org/10.1038/190372a0

Mak, W., Nesterova, T. B., De Napoles, M., Appanah, R., Yamanaka, S., Otte, A. P., & Brockdorff, N. (2004). Reactivation of the Paternal X Chromosome in Early Mouse Embryos. *Science*, *303*(5658). https://doi.org/10.1126/science.1092674

Marahrens, Y., Panning, B., Dausman, J., Strauss, W., & Jaenisch, R. (1997). Xist-deficient mice are defective in dosage compensation but not spermatogenesis. *Genes and Development*, *11*(2). https://doi.org/10.1101/gad.11.2.156

Mohandas, T., Sparkes, R. S., Hellkuhl, B., Grzeschik, K. H., & Shapiro, L. J. (1980). Expression of an X-linked gene from an inactive human X chromosome in mouse-human hybrid cells: Further evidence for the noninactivation of the steroid sulfatase locus in man. *Proceedings of the National Academy of Sciences of the United States of America*, *77*(11 I). https://doi.org/10.1073/pnas.77.11.6759

Namekawa, S. H., Payer, B., Huynh, K. D., Jaenisch, R., & Lee, J. T. (2010). Two-Step Imprinted X Inactivation: Repeat versus Genic Silencing in the Mouse. *Molecular and Cellular Biology*, *30*(13). https://doi.org/10.1128/mcb.00227-10

Nesterova, T. B., Barton, S. C., Surani, M. A., & Brockdorff, N. (2001). Loss of Xist imprinting in diploid parthenogenetic preimplantation embryos. *Developmental Biology*, *235*(2). https://doi.org/10.1006/dbio.2001.0295

Nesterova, T. B., Johnston, C. M., Appanah, R., Newall, A. E. T., Godwin, J., Alexiou, M., & Brockdorff, N. (2003). Skewing X chromosome choice by modulating sense transcription across the Xist locus. *Genes and Development*, *17*(17). https://doi.org/10.1101/gad.271203

Niwa, H., Toyooka, Y., Shimosato, D., Strumpf, D., Takahashi, K., Yagi, R., & Rossant, J.

(2005). Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. *Cell*, *123*(5). https://doi.org/10.1016/j.cell.2005.08.040

Norris, D. P., Patel, D., Kay, G. F., Penny, G. D., Brockdorff, N., Sheardown, S. A., & Rastan, S. (1994). Evidence that random and imprinted Xist expression is controlled by preemptive methylation. *Cell*, *77*(1). https://doi.org/10.1016/0092-8674(94)90233-X

Ogawa, Y., & Lee, J. T. (2003). Xite, X-inactivation intergenic transcription elements that regulate the probability of choice. *Molecular Cell*, *11*(3). https://doi.org/10.1016/S1097-2765(03)00063-7

Ohno, S., & Hauschka, T. S. (1960). Allocycly of the X-Chromosome in Tumors and Normal Tissues. *Cancer Research*, *20*(4).

Okamoto, I., Tan, S. S., & Takagi, N. (2000). X-chromosome inactivation in XX androgenetic mouse embryos surviving implantation. *Development*, *127*(19). https://doi.org/10.1242/dev.127.19.4137

Okamoto, Ikuhiro, Otte, A. P., Allis, C. D., Reinberg, D., & Heard, E. (2004). Epigenetic Dynamics of Imprinted X Inactivation during Early Mouse Development. *Science*, *303*(5658). https://doi.org/10.1126/science.1092727

Ørstavik, K. H. (2009). X chromosome inactivation in clinical practice. *Human Genetics*, *126*(3), 363–373. https://doi.org/10.1007/s00439-009-0670-5

Panning, B., Dausman, J., & Jaenisch, R. (1997). X chromosome inactivation is mediated by Xist RNA stabilization. *Cell*, *90*(5). https://doi.org/10.1016/S0092-8674(00)80355-4

Patrat, C., Okamoto, I., Diabangouaya, P., Vialon, V., Le Baccon, P., Chow, J., & Heard, E. (2009). Dynamic changes in paternal X-chromosome activity during imprinted X-chromosome inactivation in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(13). https://doi.org/10.1073/pnas.0810683106

Perry, M. (1972). Evaluation of breast tumour sex chromatin (barr body) as an index of survival and response to pituitary ablation. *British Journal of Surgery*, *59*(9). https://doi.org/10.1002/bjs.1800590912

Pillet, N., Bonny, C., & Schorderet, D. F. (1995). Characterization of the promoter region of the mouse Xist gene. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(26). https://doi.org/10.1073/pnas.92.26.12515

Plath, K., Talbot, D., Hamer, K. M., Otte, A. P., Yang, T. P., Jaenisch, R., & Panning, B. (2004). Developmentally regulated alterations in polycomb repressive complex 1 proteins on the inactive X chromosome. *Journal of Cell Biology*, *167*(6). https://doi.org/10.1083/jcb.200409026

Rougeulle, C., & Avner, P. (2003). Controlling X-inactivation in mammals: What does the centre hold? *Seminars in Cell and Developmental Biology*, *14*(6). https://doi.org/10.1016/j.semcdb.2003.09.014

Russell, L. B. (1961). Genetics of mammalian sex chromosomes. *Science*, *133*(3467). https://doi.org/10.1126/science.133.3467.1795

Russell, L. B. (1963). Mammalian X-chromosome action: Inactivation limited in spread and in region of origin. *Science*, *140*(3570). https://doi.org/10.1126/science.140.3570.976

Sado, T., Wang, Z., Sasaki, H., & Li, E. (2001). Regulation of imprinted X-chromosome inactivation in mice by Tsix. *Development*, *128*(8). https://doi.org/10.1242/dev.128.8.1275

Sheardown, S. A., Newall, A. E. T., Norris, D. P., Rastan, S., & Brockdorff, N. (1997). Regulatory elements in the minimal promoter region of the mouse Xist gene. *Gene*, *203*(2). https://doi.org/10.1016/S0378-1119(97)00507-6

Sheardown, Steven A., Duthie, S. M., Johnston, C. M., Newall, A. E. T., Formstone, E. J., Arkell, R. M., Nesterova, T. B., Alghisi, G. C., Rastan, S., & Brockdorff, N. (1997). Stabilization of Xist RNA mediates initiation of X chromosome inactivation. *Cell*, *91*(1). https://doi.org/10.1016/S0092-8674(01)80012-X

Shevchenko, A. I., Zakharova, I. S., & Zakian, S. M. (2013). The evolutionary pathway of X chromosome inactivation in mammals. In *Acta Naturae* (Vol. 5, Issue 17). https://doi.org/10.32607/20758251-2013-5-2-40-53

Tada, T., Obata, Y., Tada, M., Goto, Y., Nakatsuji, N., Tan, S. S., Kono, T., & Takagi, N. (2000). Imprint switching for non-random X-chromosome inactivation during mouse oocyte growth. *Development*, *127*(14). https://doi.org/10.1242/dev.127.14.3101

Takagi, N., & Sasaki, M. (1975). Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse. *Nature*, *256*(5519). https://doi.org/10.1038/256640a0

Tinker, A. V., & Brown, C. J. (1998). Induction of XIST expression from the human active X chromosome in mouse/human somatic cell hybrids by DNA demethylation. *Nucleic Acids Research*, *26*(12). https://doi.org/10.1093/nar/26.12.2935

Watson, J., M., J., Spencer, A., D, A., Riggs&J., ., A.Graves, & Marshall. (1992). Sex chromosome evolution: platypus gene mapping suggests that part of the human X chromosome was originally autosomalJ.M. WATSON, J.A. SPENCER, A.D. RIGGS AND J.A. MARSHALL GRAVES Proc. Natl Acad. Sci. USA 88, 11256–11260. *Trends in Genetics*, *8*(3). https://doi.org/10.1016/0168-9525(92)90192-7

Willard, H. F., Brown, C. J., Carrel, L., Hendrich, B., & Miller, A. P. (1993). Epigenetic and chromosomal control of gene expression: Molecular and genetic analysis of X chromosome inactivation. *Cold Spring Harbor Symposia on Quantitative Biology*, *58*. https://doi.org/10.1101/SQB.1993.058.01.037

Wutz, A., Rasmussen, T. P., & Jaenisch, R. (2002). Chromosomal silencing and localization are mediated by different domains of Xist RNA. *Nature Genetics*, *30*(2). https://doi.org/10.1038/ng820