ANNUAL REVIEWS

Annual Review of Animal Biosciences

Mammalian Sex Chromosome Structure, Gene Content, and Function in Male Fertility

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Annu. Rev. Anim. Biosci. 2019. 7:103–24

First published as a Review in Advance on November 9, 2018

The *Annual Review of Animal Biosciences* is online at animal.annualreviews.org

https://doi.org/10.1146/annurev-animal-020518-115332

Keywords

X chromosome, Y chromosome, dosage compensation, meiotic sex chromosome inactivation, cancer/testis antigen, male fertility

Abstract

Mammalian sex chromosomes evolved from an ordinary pair of autosomes. The X chromosome is highly conserved, whereas the Y chromosome varies among species in size, structure, and gene content. Unlike autosomes that contain randomly mixed collections of genes, the sex chromosomes are enriched in testis-biased genes related to sexual development and reproduction, particularly in spermatogenesis and male fertility. This review focuses on how sex chromosome dosage compensation takes place and why meiotic sex chromosome inactivation occurs during spermatogenesis. Furthermore, the review also emphasizes how testis-biased genes are enriched on the sex chromosomes and their functions in male fertility. It is concluded that sex chromosomes are critical to sexual development and male fertility; however, our understanding of how sex chromosome genes direct sexual development and fertility has been hampered by the structural complexities of the sex chromosomes and by the multicopy nature of the testis gene families that also play a role in immunity, cancer development, and brain function.

INTRODUCTION

In mammals, sex determination is accomplished via a chromosomal mechanism, with females having two X chromosomes and males having a single X and a single Y. The presence of the Y chromosome determines a male phenotype no matter how many copies of the X are present (1). Mammalian sex chromosomes evolved from an ordinary pair of autosomes (2). The X chromosome is highly conserved, whereas the Y chromosome varies among species in size, structure, and gene content (3, 4). Unlike autosomes that contain randomly mixed collections of genes with extremely heterogeneous patterns of developmentally regulated expression in different tissues, the sex chromosomes are enriched for sex-biased genes related to sexual development and reproduction, particularly in spermatogenesis and male fertility. In this review, I focus on the following topics: (*a*) the origin and evolution of the mammalian sex chromosomes, (*b*) sex chromosome dosage compensation, (*c*) meiotic sex chromosome inactivation (MSCI) during spermatogenesis, (*d*) the enrichment of testis-specific genes on the X chromosome, and (*e*) the gene content of the Y chromosome and its function in spermatogenesis and male fertility.

THE ORIGIN AND EVOLUTION OF THE MAMMALIAN SEX CHROMOSOMES

Muller (5) first proposed the accepted theory on the evolution of heteromorphic sex chromosomes more than a century ago. This theory has been supported by recent genetic analysis and genome sequence data (6–9). According to this theory, sex chromosomes in mammals, birds, and reptiles evolved independently from different pairs of ordinary autosomes that had an allelic difference at the sex-determining locus (10–12). These two autosomes are referred to as the ancestral protosex chromosomes (9, 13) (**Figure 1**). The proto-X and proto-Y underwent a series of addition and attrition events during evolution and became the modern X and Y (13). The therian sex chromosomes evolved ~166 Mya, whereas the human sex chromosomes diverged ~80 Mya from the eutherian ancestral sex chromosomes (14). The evolution of the therian X and Y chromosomes started from the acquisition of a gene, *SRY* (*sex-determining region Y*), on one of the protosex chromosomes, which enabled the proto-Y with a role in sex determination (**Figure 1**). A stepwise suppression of recombination between the proto-X and the proto-Y chromosome driven by *SRY* led to at least four evolutionary strata on the modern X chromosome corresponding to individual suppression events (8, 9). The suppression of recombination led to an increase in the mutation rate and in the fixation rate of deleterious mutations in the region of the Y chromosome where recombination does not occur (also known as male-specific region on the Y, or MSY). Conversely, addition of autosomal segments to both sex chromosomes occurred during the attrition process, extending the pseudoautosomal region (PAR) and contributing additional genes (**Figure 1**). Thus, the PAR is a relic of differential additions, losses, rearrangements, and degradations of the X and Y in different mammalian lineages (15). Consequently, the X chromosome is highly conserved and gene rich, and the Y chromosome has degenerated and lost most of its original genes over evolutionary time (16–18). For example, there are ~1,100 protein-coding genes on the human X (19) and <200 genes on the human Y (20). Various evolutionary models have been proposed to explain the degeneration of the Y chromosomal sequences (21–23). These include Muller's ratchet (24, 25), genetic hitchhiking (26), the Hill-Robertson effect (22, 27), and the ruby in the rubbish hypothesis (21, 28). A common feature of these models is that the efficacy of natural selection is reduced and the effective population size is decreased as a result of selective events in the MSY (21, 22, 29). The degeneration process is lineage dependent; i.e., different lineages retain different subsets of genes from the ancestral proto-Y, and Y chromosome gene content is diverse and lineage specific (9, 30,

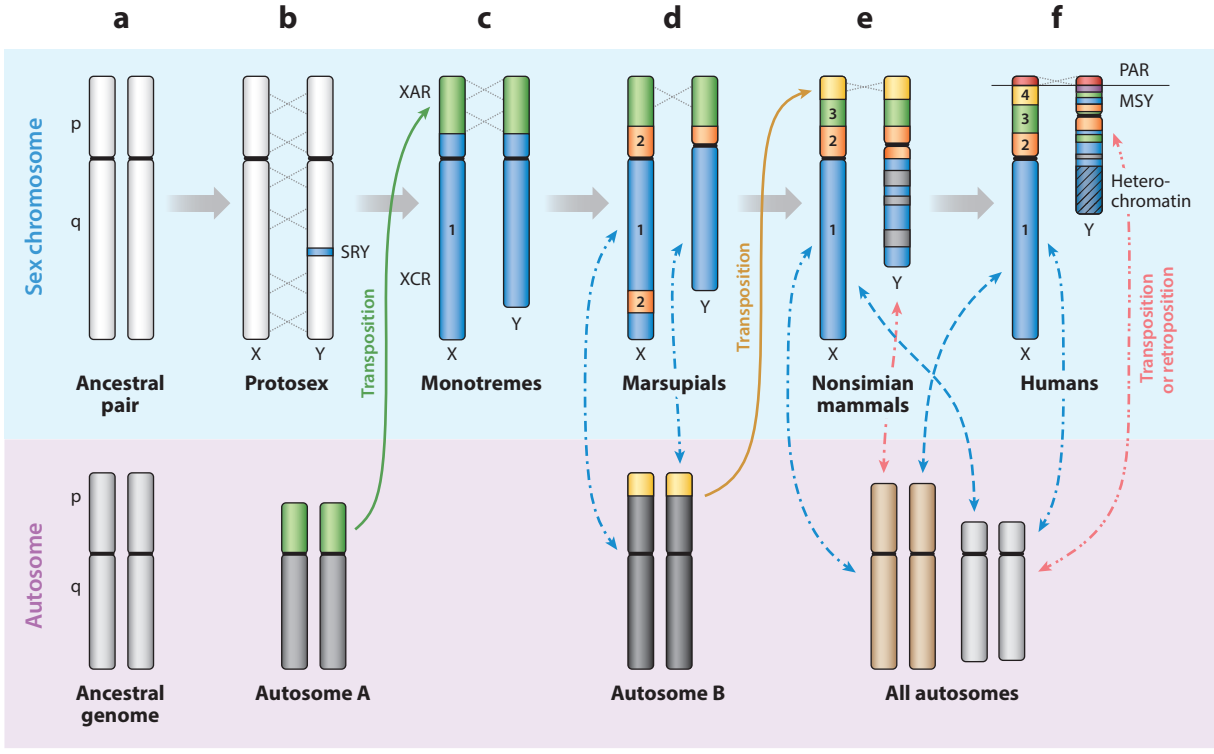

Figure 1

Evolution of the mammalian sex chromosomes and the dynamic exchange of genes between sex chromosomes and autosomes. The diagram was based on the addition–attrition model (9, 13) and the four inversions on the human Y chromosome corresponding to the four evolutionary strata found on the human X (8). (*a*) The ancestral pair of autosomes. (*b*) The protosex chromosome emerged when one partner acquired a sex-determining locus (SRY). Accumulation of male-specific alleles selected for repression of recombination (*gray crosses*), creating an X-specific region and a male-specific region on the proto-Y (MSY). (*c*) The first inversion occurred on the Y, resulting in the first evolutionary stratum (*blue*) on the X. Transposition of an autosomal segment from autosome A to the X and Y gave rise to the X added region (XAR), and the ancestral part was referred to as the X conserved region (XCR). This stage of sex chromosome is present in monotremes. (*d*) The second inversion occurred on the Y, resulting in the second stratum (*orange*) on the X. Meanwhile, attrition on the Y led to degeneration and gene loss. This stage of X and Y is present in marsupials. (*e*) The third inversion occurred on the Y, leading to the third stratum (*green*) on the X. An additional autosomal segment from a different chromosome (autosome B) added to the pseudoautosomal region (PAR) and formed the sex chromosome in nonsimian mammals. (*f*) The fourth inversion on the Y led to the fourth stratum (*yellow*) on the X and the formation of the human sex chromosome. During this process, XAR was enlarged after each addition and degraded through attrition, leaving a few functional genes and a new PAR, whereas the XCR was highly conserved (Ohno's law). The sex chromosome–to-autosome or autosome-to–sex chromosome gene transposition or retroposition events (*colored arrows*) occurred frequently during evolution, resulting in the X-originated retrogenes and X-linked cancer/testis antigen (CTX) families and the majority of the testis genes on the Y. Numbers 1, 2, 3, and 4 on the X chromosome represent the four different evolutionary strata. Abbreviations: p, short arm; q, long arm; SRY, sex-determining region Y.

31). Thus, unlike the X chromosome, which is highly conserved (2), the Y chromosome is poorly conserved among mammalian lineages in size, structural complexity, and gene content (19, 32–34).

SEX CHROMOSOME DOSAGE COMPENSATION

As a consequence of degeneration and loss of genes from the proto-Y, corresponding genes on the proto-X are left in single copy in the heterogametic sex, whereas the homogametic sex retains two copies. This leads to an imbalance of the dosage of genes on the sex chromosomes

between the sexes, relative to the autosomes (35). To equalize gene expression among males and females, different species developed unique mechanisms to achieve dosage compensation. Three main mechanisms are common to most species. One is observed in *Drosophila*, in which dosage compensation is achieved by involving twofold increased transcription of a single male X chromosome (36). The second mechanism is observed in the XX/XO system, in which sex determination is accomplished by the ratio of X chromosomes relative to autosomes, such as the one observed in *Caenorhabditis elegans*. In this system, gene expression on the X chromosome is equalized by downregulating expression of genes on both X chromosomes of hermaphroditic XX organisms by half (37). The third mechanism is seen in mammals, in which one of the two X chromosomes in females is randomly inactivated during early embryonic development, resulting in one actively transcribed X chromosome in every female somatic cell to balance the single X in the male genome. The inactive X chromosome forms a discrete body within the nucleus called a Barr body (38, 39). In the female germline, however, the inactive X is reactivated before meiosis, which ensures that all oocytes will inherit an active X chromosome (40, 41) (**Figure 2**).

Upon completion of X inactivation in females, how does the expression of X-linked genes (which have one active allele) remain balanced to the expression of autosomal genes (which have two active alleles)? An earlier hypothesis put forward by Ohno (1) in 1967 predicted that the X-linked genes are expressed at twice the level of autosomal genes per active allele to regain dosage balance. This hypothesis has been well accepted and supported by gene expression data from microarray analysis (42–45). However, Ohno's hypothesis has been challenged in recent years by genome-wide transcriptomic (RNA-seq) and proteomic analysis, in which a gene expression ratio of ~0.5 between the X and autosomes was found among 12 human and 3 mouse tissues

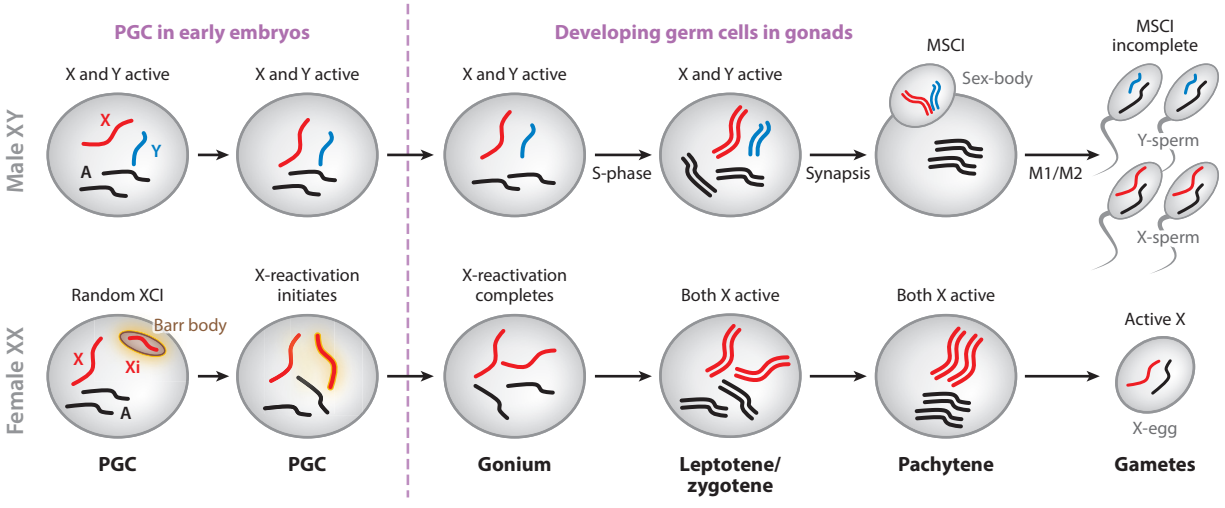

Figure 2

The behavior and activity of mammalian sex chromosomes during male and female germ cell development. During spermatogenesis, spermatocytes undergo two rounds of meiosis to produce four sperm, and each sperm has an X (or a Y) chromosome and a set of autosomes. The X and Y chromosomes remain active until pachytene, when meiotic sex chromosome inactivation (MSCI) occurs and sex-body is present. MSCI is incomplete in the postmeiotic germ cells. Female primordial germ cells (PGCs) in the early embryo have one active X and one inactive X chromosome (Xi) called the Barr body. The Xi reactivation initiates during migration of PGCs to the genital ridge and completes after colonization of the genital ridge. Throughout meiosis and in postmeiotic germ cells, both X chromosomes remain active. Each egg contains a set of autosomes plus an active X chromosome. Figure adapted from Heard & Turner (56) with permission from Cold Spring Harbor Laboratory Press. Abbreviation: XCI, X chromosome inactivation.

(46). The ratio of ~0.5 is observed in both males and females and applies equally to X-linked genes that emerged before and after the evolution of the X chromosome (46). This study raises a fundamental question about the dosage compensation mechanism in mammals. As genome-wide transcriptomic and proteomic analysis has become routine in most species, it is interesting to see that recent studies have yielded some more surprising results concerning dosage compensation in animals (47, 48). At least four different types of dosage compensation have been observed, as Gu & Walters (47) recently described: (*a*) complete compensation with balance (X = XX = A), (*b*) incomplete compensation with balance (X = XX < A), (*c*) incomplete compensation without balance (X < XX = A), and (*d*) complete compensation without balance (X = A < XX). Here, A refers to either diploid autosomal or ancestral expression levels, depending on the empirical approach used in the analysis (e.g., comparative method or X-to-autosome) (47). A recent study on the microRNA (miRNA) target sites in X- and Y-linked genes revealed that the conserved miRNA target sites in mammals unveil preexisting heterogeneities in gene dosage sensitivities that shaped mammalian sex chromosome evolution (49). Although the debate over the status and mechanism of dosage compensation in mammals continues, there is an urgent need to standardize the methods used in genomic and proteomic data processing and analysis, as well as to standardize the terminology related to sex chromosome dosage compensation (48, 50).

MEIOTIC SEX CHROMOSOME INACTIVATION DURING SPERMATOGENESIS IN MALES

Research on sex chromosome dosage compensation in reproductive organs is problematic because gene expression in gonads (germ cells) differs dramatically from that in somatic tissues. At least three major factors contribute to the distinctive patterns of gene expression in gonads (47). One is that mechanisms mediating dosage compensation often may not operate in the gonads. Another one is unequal complement of sex-biased genes on the sex chromosomes relative to autosomes, reflecting the fact that sex chromosomes are a hot spot for sexually antagonistic evolution (see below; also see the review in 47). The third factor is MSCI in males, which is the process of transcriptional silencing of the sex (X and Y) chromosomes that occurs during the meiotic phase of spermatogenesis, observed in mammals and other lineages (51–53). MSCI is essential for male fertility, and errors in MSCI, such as the escape of the X or Y chromosome from MSCI, are usually associated with meiotic arrest and impaired fertility (52, 54–56).

During the early stages of spermatogenesis, spermatogonial stem cells undergo mitotic divisions into spermatogonia and primary spermatocytes, where genes on the X and Y chromosomes are transcriptionally active. It is believed that MSCI occurs shortly after the zygotene-to-pachytene transition, when meiotic synapsis between homologous pairs of autosomes is complete. The X and Y chromosomes, however, can synapse only at the small distal PAR; outside of PAR, they remain unsynapsed. This asynapsis triggers MSCI. As a result, the X and Y chromosomes become transcriptionally silenced and compartmentalized into a peripheral nuclear subdomain called the sex-body (or XY-body) (57) (**Figure 2**). Although the reason for the transcriptional silencing remains unclear, it is thought to prevent recombination between nonhomologous sex chromosomes and also to act as a quality-control mechanism to selectively eliminate germ cells with synaptic defects (52, 58).

MSCI is considered as a special example of a more general mechanism called meiotic silencing of unsynapsed chromatin (MSUC), which silences any chromosomes that fail to pair with their homologous partners and prevents aneuploidy in subsequent generations (52, 59). Although the mechanisms underlying MSCI/MSUC are not completely understood, recent studies have revealed that extensive chromatin modifications, including histone methylation, ubiquitylation, and

deacetylation, occur on the X and Y that result in significant downregulation of genes on the sex chromosomes (60). Similar to the somatic X chromosome inactivation (XCI), the expression of two long noncoding RNA, called *Xist* (inactive X specific transcript) and *Tsix* (antisense transcript of *Xist*), located on both the X and Y chromosomes, initiates the transcriptional silence (61–63). In males, *Xist* is expressed exclusively in the testis, and the *Xist* RNA coats the entire sex-body (53, 64). However, the deletion of the mouse *Xist* gene, which interrupted the dosage compensation, did not disturb the formation of the XY-body and the initiation of MSCI in germline, suggesting that *Xist* is dispensable for MSCI (65, 66). Thus, MSCI may be regulated by a different mechanism in the male germ cells than XCI in the female somatic cells. Indeed, Turner and colleagues have shown that MSCI is regulated by coating the largely unpaired meiotic X-Y bivalent with a tumor suppressor protein BRCA1 (BRCA1, DNA repair associated). The latter attracts the ATR (ataxia telangiectasia and Rad3 related) kinase that phosphorylates H2AX (histone family, member X), leading to condensation of chromatin on the X and Y chromosomes in primary spermatocytes (52, 67). Additionally, in a detailed comparison between XCI and MSCI, Yan & McCarrey (68) revealed that approximately 15–25% of X-linked protein-coding genes (mRNAs) escape XCI, whereas none of the X-linked protein-coding genes escape MSCI. In contrast, more than 80% of X-linked miRNAs escape MSCI, whereas no miRNA appears to escape XCI. Hence, some of the X-linked miRNA genes that escape MSCI may encode miRNAs that play a regulatory role in MSCI (68, 69).

How long MSCI persists during spermatogenesis has been subject to ongoing debate and may vary among species. It is generally believed that the X and Y chromosomes become transcriptionally repressed from pachytene onward, throughout meiosis, and to the end of spermatogenesis (52, 54, 70), although MSCI is found incomplete in the postmeiotic germ cells (71). If so, one would predict that most X- and Y-linked genes that are essential for meiosis and spermiogenesis would be transcriptionally shut down with a potential interruption in spermatogenesis. To avoid this consequence, two independent systems have evolved in male germ cells to tolerate MSCI. One is the X-linked gene backup system, where genes carrying out essential metabolic functions integrate at autosomal sites through a retroposon-mediated X-to-autosome gene transposition event (72). More than 20 X-originated retrogenes have been reported, and the majority (70%) of them have testis-specific expression at the initiation of MSCI (72), thus compensating for the silencing of X-encoded products. The loss of function of one of these retrogenes, *Utp14b* (UTP14B small subunit processome component), causes spermatogenic failure (73, 74), signifying the importance of this backup system for male spermatogenesis. The other system is the development of the male germ cell–specific organelle, known as the germinal granule, which includes intermitochondrial cement (IMC) and chromatoid body (CB) (75, 76). IMC is found in the cytoplasm of spermatocytes and disappears during meiosis, whereas CB is present in the cytoplasm of postmeiotic spermatids. CB may have originated from the dense material of IMC (77, 78). Previous studies on the rodent CB revealed that it moves around the cytoplasm, making contact with other subcellular organelles, such as the nucleus, mitochondria, and the Golgi apparatus, and passes through cytoplasmic bridges of sister spermatids (79, 80). The transcription products, including messenger RNAs (mRNAs), miRNAs, and Piwi-interacting RNAs (piRNAs), are initially transported into CBs from the nucleus and stored there. In addition, CB also contains RNA-binding proteins, RNA helicases, and other proteins involved in various RNA regulatory pathways (76). Thus, CB is a center for posttranscriptional mRNA storage and regulation and small RNA–mediated regulation of gene expression in haploid male germ cells (76, 81) when MSCI persists during spermiogenesis. Recent studies on a mouse X-linked gene, *Prame* (preferentially expressed antigen in melanoma), and a bovine Y-linked gene, *PRAMEY* (preferentially expressed antigen in melanoma, Y-linked), have revealed that these genes are expressed before meiosis and are enriched in CBs involved in

spermiogenesis (78, C. Lu, R. Mossi, A. Wang, F. Diaz, and W.-S. Liu, manuscript submitted). In addition, a large group of X-linked multicopy gene families on the human and mouse X chromosomes (see discussion below) are found to be expressed predominantly in postmeiotic cells, indicating that MSCI is incomplete during spermiogenesis (71, 82). It remains to be seen if the incomplete MSCI is a common phenomenon in other species.

Both the X-linked gene backup system and the germinal granules are largely consistent with an earlier hypothesis of haploid syncytium (83, 84). This hypothesis focused on the nonequality of sex chromosome constitution in haploid germ cells and predicted that the genetically haploid spermatids are phenotypically diploid (83). During spermatogenesis, spermatocytes undergo two rounds of meiosis to produce four sperm. Each sperm contains either an X or a Y chromosome and one set of autosomes (**Figure 2**). During both the mitotic and meiotic cell divisions, cytokinesis is incomplete, giving rise to syncytia of cells interconnected by stable intercellular bridges. The intercellular bridges connecting the cells permit the sharing of cytoplasmic constituents, thus ensuring the synchronous development of a clone of cells and gametic equivalence between haploid spermatids (83). As mentioned above, CB moves freely across the intercellular bridges and serves as a vehicle to move gene transcripts and proteins among the interconnected haploid spermatids. This sharing of cytoplasmic constituents may not be sufficient for Y-bearing spermatids to acquire the same amount of products of some X-linked genes as the X-bearing spermatids, signifying the need for the X-linked gene backup system, in which the X-originated retrogenes provide an equal amount of their product for both X- and Y-bearing spermatids during spermatogenesis (72, 85, 86).

SEX- AND REPRODUCTION-BIASED GENE CONTENT OF THE MAMMALIAN X CHROMOSOME

Theoretically, each chromosome in a genome should contain randomly mixed collections of genes with extremely heterogeneous patterns of developmentally regulated expression in different tissues. This is true for all autosomes, but not for the sex chromosomes in mammalian genomes. The gene content of the sex chromosomes is strikingly different from that of the autosomes. Both sex chromosomes appear to be enriched for genes related to sexual differentiation, brain development, and reproduction (19, 20, 71, 87–91). It should be noted that the generally accepted random nature of autosomal gene order may not actually be correct, as the genome appears to have a hierarchical spatial orientation that facilitates coordinated gene regulation (92).

X Chromosome Gene Acquisition and the Violation of Ohno's Law

Ohno's law states that the mammalian X chromosome is highly conserved in its size (approximately 5% of the genome) and gene content (2). In humans, the X chromosome is 155 Mb in size, representing approximately 5% of the haploid genome, and bears ~1,100 protein-coding genes (19), roughly 75% of which are single-copy genes while the remaining genes are in multicopies. A recent comparative study between the human and mouse X chromosomes indicated that 95% of human X-linked single-copy genes are conserved with the mouse X chromosome, well in line with Ohno's law. These single-copy genes are expressed in both sexes. However, a total of 144 X-linked genes in humans and 197 X-linked genes in mice are found to be exceptions to Ohno's law (82). The genes that violate Ohno's law fall into three groups: Group I includes genes that are the outcome of lineage-specific gene loss (55/144 in humans and 34/197 in mice). Group II contains the majority of genes exceptional to Ohno's law (76/144 in humans and 134/197 in mice). These genes are located mainly in the X ampliconic regions, which comprise ~2% of the

X chromosome DNA, and were independently acquired in different lineages during evolution. These genes are amplified in multicopies and are predominantly expressed in testis (see section titled X-Linked Cancer/Testis Antigens below). In contrast to the X-to-autosome gene retroposition (72), these X-linked multicopy gene families are essentially originated through an autosome-to-X gene transposition or retroposition mechanism (**Figure 1**). Group III contains a small portion of the genes that violate Ohno's law (13/144 in humans and 29/197 in mice), which are derived through duplication of an ancestral X-linked gene (82). Since the divergence of the human and mouse lineages from the common ancestor 80 Mya (93), 55 and 34 X-linked genes have been lost in a lineage-specific manner, whereas 76 and 134 gene families have been independently acquired in humans and mice, respectively (82). These data suggest that the mammalian X chromosome is under rapid evolution.

The X Chromosome Is "Sexy and Smart"

Compared with autosomes, the mouse X chromosome harbors threefold more genes that are expressed in female reproductive organs, including ovary and placenta, and 15-fold more male germ cell–related genes specifically expressed in the testis (90, 91, 94). In addition, more than 200 X-linked genes are expressed in the human brain, with a function in brain development and cognitive abilities. These intelligence genes are six-fold in excess on the X (9, 95). Therefore, the X is considered the "sexy and smart" chromosome of the mammalian genome (9, 96).

How is the ancestral protoX evolved into the modern "sexy and smart" X chromosome? This question could be addressed by the joint actions of sexual antagonism (or sexual conflict) theory and the faster-X adaptive evolution model. Sexual antagonism occurs when the two sexes have conflicting optimal fitness strategies concerning reproduction, potentially leading to an evolutionary arms race between males and females. When selection differs between the sexes, a mutation beneficial to one sex may be harmful to the other (97–99). Based on this theory, Rice (100) predicted that genes involved in spermatogenesis should be enriched on the X chromosome. A recessive mutation in an X-linked gene that conferred a reproductive advantage in males but was deleterious to females would spread rapidly in a population, because it would be immediately noticeable phenotypically in males owing to its hemizygous state. Eventually, females would arise that are homozygous for the mutation. It would impair their reproductive capacity and, in turn, lead to the evolution of modifiers that restrict the expression of the gene to the testis (60). The X chromosome is also expected to be enriched in genes involved in oogenesis (100), as the X chromosome spends two-thirds of its evolutionary time in females, thus increasing the chance that a mutation conferring a female reproductive advantage will become fixed (60).

In the faster-X evolution model, the X chromosome is predicted to have higher rates of adaptive evolution than autosomal loci if beneficial mutations are on average recessive, because selection will act more efficiently on X-linked mutations exposed in hemizygous males (101). Under this model, faster-X evolution should occur more often on male-specific genes (100). This is supported by recent genome-wide transcriptomic and proteomic analyses (101, 102). By analyzing genome-wide nucleotide polymorphisms and nucleotide divergence between the mouse and rat genome, Kousathanas and colleagues (102) compared rates of adaptive evolution for autosomal and X-linked protein-coding genes. They found significantly faster adaptive evolution for X-linked genes, particularly for those genes that are expressed in testis and that escape MSCI (102). Furthermore, analysis on spermatogenic cell–specific transcriptome profiles in two subspecies of mice indicated that faster-X protein evolution occurs at all stages of spermatogenesis. In contrast, faster-late protein evolution was found for both X-linked and autosomal genes (101). This study also identified a slower-late pattern, referring to the X-linked and testis-expressed autosomal genes that share a

common feature of less expression divergence late in spermatogenesis. The slower-late expression divergence reflects that strong regulatory constraints are imposed during this critical stage of sperm development and that these constraints are particularly stringent on the tightly regulated sex chromosomes (101).

The reason for the accumulation of intelligence genes on the X chromosome is still unclear. An earlier study identified a total of 958 entries related to "mental retardation" in the Online Mendelian Inheritance in Man database (95), of which 215 refer to X-linked mental retardation (103). This phenomenon is referred to as the "large X chromosome effect" (102, 104–106), a consequence of sexual selection (95, 107). One sexual selection model proposed by Lande & Arnold (108) focused on the joint development of the male sexual characteristic and the female mating preference in the same genetic system. This model describes the male sexual characteristic as a specific behavioral trait (not as a morphological trait) and proposed that female mating choice has been the dominant factor to shape the human mind during evolution. In this system, there is a positive feedback in both sexes on the same genes responsible for development of secondary sexual characteristics. Such a positive feedback process in both sexes may be a major driver for the remarkably fast development of human brain function. This model is reflected by two facts: the rapid (threefold) increase in human brain size in the past 2.5 million years (95) and the tight association of gene expression between brain and testis, also known as "brains and balls" coincidence (9). In the latter case, apart from the predominant expression in the brain, many of these X-linked intelligence genes are also expressed in the testis. The X-linked intelligence genes may also contribute to increased male fertility (95).

Speciation is the process by which new biological species arise, corresponding to the evolution of reproductive barriers that limit the potential for genetic exchange between populations (106). Because of its predisposition to accumulate genes that affect reproduction and cognitive function, the X chromosome provides pre- and postmating reproductive barriers that are critical to mammal speciation. Thus, the "sexy and smart" X chromosome has been considered "the engine of speciation" (9, 96, 106, 107).

X-Linked Cancer/Testis Antigens

The cancer/testis antigens (CTAs) are a group of proteins that are typically expressed in almost all types of cancer cells and in normal male germ cells in testis but are silent in almost all somatic cells. Because of this unique expression pattern, the CTAs are considered attractive targets for cancer biomarkers and immunotherapy (109, 110). To date, a total of 276 CTAs have been identified in the human genome (**http://www.cta.lncc.br/**), of which 128 (46.4%) map to the X chromosome (termed CTXs), 9 (3.3%) map to the Y chromosome, and the remaining 139 (50.3%) are distributed on the autosomes. It is remarkable that as many as 11.6% (128/1,100) of the genes on the X chromosome belong to CTX families, whereas only 0.8% (139/17,900) of genes on autosomes (assuming that there are a total of 19,000 protein-coding genes in the human genome) (111) are CTAs. Therefore, the X chromosome has a 15-fold enrichment of CTA genes over the autosomes (90, 94).

Among the 128 CTX antigens, 22 are single-copy genes, while the remaining 106 belong to 12 multicopy gene families (**Table 1**). Gene copy number varies among the CTX families, ranging from 2 members in CT7 (*MAGEC1*) to 18 members in CT4 (*GAUGE*). However, the largest CTA family, *PRAME*, has an estimated 37–50 members and maps to autosomes (chromosome 1 and 22) in human (109, 112). Approximately 90 and 60 members of *PRAME* were identified in the mouse and bovine genome, respectively. Remarkably, *PRAME* has been transposed to the

Table 1 CTX gene families on the human X chromosome[a]

CT identifier	Gene family symbol	Gene family name	Gene copy number	Location on X
CT1	*MAGEA*	Melanoma antigen gene family A	14	Xq28
CT3	*MAGEB*	Melanoma antigen gene family B	6	Xp21.3
CT4	*GAUGE*	G antigen	18	Xp11.4-p11.2
CT5	*SSX*	Sister-of-Sex-lethal (or SSX family)	10	Xp11.23-p11.22
CT6	*CTAG1*	Cancer/testis antigen 1 (or NY-ESO-1)	3	Xq28
CT7	*MAGEC1*	Melanoma antigen gene family C	2	Xq26-q27
CT11	*SPANX*	Sperm protein associated with the nucleus, X-linked (family A, B, C, D, N)	13	Xq27.1-q27.3
CT12	*XAGE*	X antigen	11	Xp11.22-p11.21
CT16	*PAGE5*	P antigen family member 5	7	Xp11.23-p11.21
CT24	*CSAG1*	Chondrosarcoma-associated gene 1	3	Xq28
CT45	*CT45*	Cancer/testis antigen family 45	6	Xq26.3
CT47	*CT47*	Cancer/testis antigen family 47	13	Xq24

[a]Data taken from the CT Database (**http://www.cta.lncc.br/**) and National Center for Biotechnology Information (**https://www.ncbi.nlm.nih.gov/**).

X chromosome in rodents and the Y chromosome in bovids during evolution (3, 112–114) (see section titled Testis Genes on the Y Chromosome below).

As mentioned above, these CTXs belong to the Group II genes that are exceptions to Ohno's law. They originated primarily in a lineage-specific manner via the autosome-to-X gene transposition (or retroposition) and amplified thereafter during evolution (82). A comparison between human and chimpanzee CTXs indicated that these X-linked gene families have been conserved (but with variations in copy number in some CTX families) since the divergence of the two species approximately 6 Mya (109, 115). However, the sequence similarity of CTX genes between human and chimpanzee is significantly lower than that of the autosomal (or X-linked non-CTX) genes. In molecular evolution, the ratio of the nonsynonymous-to-synonymous mutation rate (dN/dS) indicates the type of evolutionary pressure acting on a protein-coding gene. $dN/dS < 1$ indicates negative (or purifying) selection; $dN/dS = 1$ indicates neutral evolution; and $dN/dS > 1$ indicates positive selection (116). The dN/dS ratios of CTXs are significantly higher than those of X-linked non-CTX genes ($P = 2.3 \times 10^{-10}$) (109). Many CTXs have an excess of ratios >1; for instance, *MAGEA*, *MAGEB*, *GAUGE*, *SSX*, *XAGE*, *PAGE5*, and *CSAG1* gene families have a ratio >2, indicating a strong positive selection on the CTX gene products (109). Therefore, the CTX genes are a foundation for the development of the faster-X evolution model (101, 102).

Despite the progress in human CTX studies, no detailed analysis has been carried out on CTXs in other lineages. Emerging evidence indicates that the large group of faster-X genes reported recently on the mouse X chromosome are likely to be CTXs (82, 101, 107). Among the 12 human CTX gene families listed in **Table 1**, only *MAGE* (A, B, C) and *CT47* are conserved on the mouse X chromosome. In a BLASTp search comparing the human CTXs against the bovine genome sequence, a total of 86 proteins corresponding to 18 CTX families were retrieved, including *MAGE* (A, B, D, E) and *CT47* (117), suggesting these two CTX families originated from the protosex chromosome.

Like other CTAs, CTXs have been studied extensively in cancer biology and are frequently associated with advanced disease with poorer prognosis (109, 118–120). The significance of this

correlation is unclear because the functions of the CTAs/CTXs in the disease process remain poorly understood. A recent study revealed that a majority of CTAs/CTXs are intrinsically disordered proteins (IDPs) (121). By definition, IDPs are proteins that lack a stable 3D structure, and they are often associated with dosage sensitivity (see section titled Sex Chromosome Dosage Compensation above) (121). IDPs appear to function in several important cellular processes, such as transcriptional regulation, signal transduction, and cell growth (121, 122). Emerging evidence indicates that epigenetic modifications, in particular demethylation, play an essential role in CTA/CTX regulation. In cancer cells, de-repression of CTA/CTX genes is largely explained by demethylation in their promoter region (121, 123).

Apart from the fact that CTXs are predominantly expressed in postmeiotic cells in the testis and violate Ohno's law (71, 82), the function of CTXs in germ cell development and male reproduction is largely unknown. Several CTXs are associated with maintaining the undifferentiated state of stem cells. For example, *SSX* and *CTAG1* (*NY-ESO-1*) are expressed in undifferentiated mesenchymal stem cells (124), and *GAUGE* and *MAGEA* are expressed in embryonic stem cells during early human development and may play a role in migrating primordial germ cells (125, 126).

As discussed earlier, the largest CTA family identified to date is PRAME (112), which was first discovered in human melanoma cells (127). PRAME is expressed at high levels in testis and in a wide variety of malignant tumors (128, 129), and it has been used as a prognostic marker for patients with various cancers (130–132). Some members of PRAME are also expressed in other reproductive tissues, such as ovary (oocytes), placenta, and endometrium (127, 133–136). One of the X-linked *Prame* in the mouse genome is expressed in spermatogenic cells and mature sperm, particularly in the acrosome and sperm tail. A conditional *Prame* knockout (cKO) line has been developed recently, and male cKO mice have a significantly smaller testis and decreased sperm count but normal fertility (C. Lu, R. Mossi, A. Wang, F. Diaz, and W.-S. Liu, manuscript submitted). Although there are great challenges in determining the functional role of each member in a given CTX family, I believe we will not fully understand spermatogenesis and male fertility until we know the functions of all CTXs.

TESTIS-BIASED GENE CONTENT OF THE MAMMALIAN Y CHROMOSOME

The mammalian Y chromosome is usually the smallest chromosome of the genome, comprising <3% of the haploid genome (137). The PAR is a small region (~5% of the Y) typically located in the distal part of either the short or long arm (Yp or Yq), where X and Y chromosomes pair and recombine during meiosis. The rest of the Y (~95%) is the MSY, which does not recombine with the X during meiosis (17). Several special features set the Y chromosome apart from the rest of the genome: male-limited transmission, absence of recombination, abundance of Y-specific repetitive sequences, degeneration of Y-linked genes during evolution, acquisition of autosomal genes, and accumulation and functional cluster of testis genes for maleness and fertility (23). The absence of recombination between the X and Y chromosomes makes classical linkage mapping of MSY virtually impossible, and the complexity of the repetitive sequences makes the Y chromosome a difficult target for sequencing (88, 138). That is why the Y has been excluded from most mammalian genome sequencing projects. Our knowledge regarding the mammalian Y chromosome is based largely on the sequenced data from primate (human, chimpanzee, rhesus macaque, and gorilla) (7, 20, 139–141), mouse (142), and bovine (3) Y chromosomes.

In contrast to the X chromosome, which has been shaped by dosage differences and faster adaptive evolution to have a biased gene content, the Y chromosome seems to be a product of an

evolutionary process that has led to its degradation and loss of over 98% of its original set of ancestral genes on the proto-Y (9). Questions regarding how the Y has been degenerated, as well as the possible mechanisms, have been addressed in a review article by Graves (9) and are not discussed here. In contrast to the Y chromosome degeneration theory, a recent study revealed that Y-to-autosome transposition (or retrotransposition) could be a common mechanism underlying Y chromosome gene loss in mammals (143). Similar to the X-to-autosome retrotransposed genes (72), the Y-originated retrogenes, such as *EIF1AY*, *EIF2S3Y*, *RPS4Y*, and *UBA1Y*, are important for spermatogenesis. This has been proposed as a compensatory mechanism to balance MSCI (143).

Is the Y Chromosome Gene Poor or Gene Rich?

Before the 1950s, the Y was considered as a genetic desert or functional wasteland. In 1959, reports of XO females and XXY males established the existence of a sex-determining gene on the human Y chromosome (144–146). The *SRY* gene was discovered in 1990 (147). The presence of *SRY* is sufficient to initiate the male developmental program in the vast majority of mammalian species (148). For many years, the Y chromosome was considered to have the sole function of triggering testis development (2, 149), until the discovery in the 1990s of Y chromosome (micro)deletions that cause spermatogenic failure and low/subfertility in males (150, 151).

Sequencing of the human Y chromosome, which has been restricted to the 23-Mb MSY euchromatic region because of difficulties associated with the heterochromatic region of MSY, identified 27 protein-coding genes/families, including 18 single-copy genes and 9 gene families. Together, the human MSY harbors a total of 78 protein-coding genes (20). Therefore, the gene density on the human MSY is 3.4 genes/Mb (78/23), relatively lower than those on the X chromosome (1,100/155 = 7.1) and autosomes (17,900/3,200 = 5.6). The gene density on the chimpanzee, Rhesus macaque, and gorilla Y chromosomes is similar to that of the human Y (7, 20, 140, 141). Thus, the primate Y chromosome is gene poor. A widely accepted hypothesis suggests that the other mammalian Y chromosomes are also heterochromatic, gene poor, and transcriptionally inert. However, this hypothesis has been challenged by recent gene annotation on the mouse and bovine Y chromosomes (3, 152).

The mouse Y is ~90 Mb in size, is essentially euchromatic, and contains 9 single-copy and 8 multicopy gene families. The copy number varies among these families, ranging from 2 in *Zfy* to 221 in *Sty2*. Together, the mouse Y has 674 protein-coding genes. Therefore, the mouse Y is not gene poor, though its gene density (7.5 genes/Mb) is slightly lower than that of the mouse X (9 genes/Mb) and autosomes (11.5 genes/Mb) (152).

The bovine Y chromosome is estimated to be 51 Mb. Similar to the mouse Y, the bovine MSY (~41 Mb) is euchromatic. The annotation of the bovine MSY identified a total of 1,274 protein-coding genes belonging to 28 gene families, including 12 single-copy genes and 16 multicopy families. The copy number varies among these families, ranging from 3 in *EGLY* to 234 in *ZNF280BY*. The average gene density on the bovine MSY is ~31.2 genes/Mb (1,274/41), which is threefold higher than those of the bovine X chromosome (9.4 genes/Mb) and autosomes (10.2 genes/Mb). Therefore, the bovine Y chromosome is relatively gene rich (3).

How Many Proteins Do Mammalian Y Chromosomes Encode?

Regardless of the gene-poor or gene-rich status of the Y, the human, mouse, and bovine Y each encode <30 proteins (or protein families). The question is therefore how many proteins are encoded by the Y chromosomes of mammals. Through a comparative mapping approach with genome-wide transcriptome data, Cortez et al. (153) identified a total of 134 different

protein-coding genes among 15 mammals, including 2 monotremes (platypus and echidna), 2 marsupials, and 11 eutherian species (human, chimpanzee, gorilla, orangutan, Rhesus, marmoset, mouse, rat, cat, dog, and elephant). Their results suggest that the mammalian Y chromosomes will collectively have a small number of protein-coding genes (families), maybe just in hundreds.

In general, there are two groups of protein-coding genes on the Y: single-copy and multicopy genes. These two groups have distinct features with different functions. The single-copy genes are located in the X-degenerated regions, which are the relics of the ancestral protosex chromosome. They have an X-linked counterpart, although they do not recombine during meiosis. A recent study on single-copy genes among 8 mammals (human, chimpanzee, rhesus, marmoset, mouse, rat, bull, and opossum) identified a total of 36 ancestral X-Y shared gene pairs (6). These genes are broadly expressed, with regulatory functions in transcription, splicing, translation, ubiquitination, and chromatin modification, suggesting the Y chromosome has an impact on gene regulation across the genome in males, potentially influencing biological functions throughout life and in every tissue (154, 155). In contrast, the Y-linked multicopy gene families are very similar to the X-linked ampliconic genes in their origin. They were primarily independently acquired in different lineages during evolution and are exclusively expressed in testis, with a function in spermatogenesis and male fertility.

Testis Genes on the Y Chromosome

With a few exceptions (such as *TSPY* and *HSFY*), almost all Y-linked multicopy genes originated through a lineage-specific autosome-to-Y gene transposition or retroposition. After the initial transposition or retroposition, the transposed gene is usually amplified to survive in a harsh environment, i.e., lacking a gene recombination and repair system. A common feature identified from the human, mouse, and bovine MSY ampliconic regions is the palindrome sequences, which harbor all of the multicopy gene families. These palindromes exhibit intrachromosomal identities of 99.9% or greater (20) and are believed to play an essential role in conserving Y gene functions across evolutionary time through a mechanism of Y-to-Y gene conversion (156).

The human, chimpanzee, and gorilla MSY have 8, 19, and 21 palindromes, respectively, whereas the rhesus MSY has only 3 small palindromes. Unlike in humans, most palindromes in chimpanzees exist in multiple copies so that each palindrome arm has multiple potential partners for both intra- and interpalindrome gene conversion (21). The human ampliconic region contains 9 gene families, with a copy number of 2 for *VCY*, *XKRY*, *HSFY*, and *PRY*; 3 for *BPY2*; 4 for *CDY* and *DAZ*; 6 for *RBMY*; and ~35 for *TSPY*. All are expressed predominantly or exclusively in testes (20). Out of 9 gene families present in humans, one (*XKRY*) becomes a pseudogene, and two (*HSFY* and *PRY*) are absent in chimpanzees (7). Thus, the gene repertoire of the chimpanzee MSY is much smaller and simpler than that of the human MSY. In addition, the human ampliconic region also contains 78 putative noncoding transcription units that also are expressed predominantly in testes (20).

Compared with the primate MSY, the mouse MSY is significantly larger (89.6 Mb) (152) and contains ~200 large repeats. These repeats are composed of two different types: regular and prevalent 515-kb tandem repeats (142) and less regular, less prevalent 400-kb palindromes, which are clustered along the length of MSY (152). Each repeat typically contains four protein-coding gene families, including *Sly*, *Srsy*, *Ssty1*, and *Ssty2*, with estimated copy numbers of 126, 197, 85, and 221, respectively (152). These genes are expressed exclusively in testes, and deletion of the mouse MSY (i.e., reduced copy numbers of these genes) leads to sperm abnormalities and subfertility (142, 157–160).

In the bovine MSY, the majority (69%) of the ampliconic sequences are palindrome-like and share intrachromosomal similarities >99%. An estimated ~80 repeat units are present on the

sequenced Y chromosome of the Hereford bull (L1 Domino 99375) (3). Each repeat unit is ~420 Kb and contains four gene families, *HSFY*, *TSPY*, *ZNF280BY*, and *ZNF280AY*, with estimated copy numbers of 190, 136, 234, and 79, respectively (3). In addition to the X-degenerated and Y-ampliconic regions, there is a Y-transitional region in the bovine MSY, where the single-copy genes and the multicopy *PRAMEY* gene family are located (3). Copy number variations of *TSPY*, *HSFY*, *ZNF280BY*, and *PRAMEY* are associated with sperm/semen quality and male fertility (3, 161–165).

In contrast to the highly enriched CTAs on the human X chromosome (90, 94), only one CTA gene family, *TSPY*, has been found on the human Y chromosome (**http://www.cta.lncc.br/**). *TSPY* is conserved and is arranged in tandemly repeated clusters on the Y chromosome of several other placental mammals, including cattle, goats, sheep, horses, and rodents (166). In addition to TSPY, another CTA, termed PRAMEY, is found on the bovine Y chromosome (112). Like the mouse X-linked *Prame* gene product (C. Lu, R. Mossi, A. Wang, F. Diaz, and W.-S. Liu, manuscript submitted), the bovine PRAMEY is a novel male germ cell–specific and germinal granule-associated protein that is expressed in spermatogenic cells, particularly in CB and acrosome. Although the function of PRAMEY during spermatogenesis remains unclear, these results suggest that PRAMEY may play an essential role in acrosome biogenesis and spermiogenesis (78).

The Y Chromosome and Male Infertility

Subfertility and infertility are common problems in human and other mammals. Infertility affects an estimated 15% of couples of reproductive age, and human male infertility frequently appears to be idiopathic. Among all genetic factors that contribute to human spermatogenic failure and infertility, Y chromosome microdeletion is the most significant identified to date (150, 167, 168). Because many review articles have been written on the subject of the Y chromosome and infertility (137, 169–171), this is not the focus of the current review. However, it is worth noting that, besides association of Y chromosome microdeletions, Y haplotypes, and copy number variations of Y-linked multicopy gene families with subfertility and infertility in human or semen quality and male reproduction in livestock, such as in cattle (161, 164), the functional role of almost all Y-linked testis gene families and the underlying molecular mechanisms are still mysterious and need to be addressed.

CONCLUSION

Sexual reproduction begins with the male and female gametes. In mammals, males produce X-bearing and Y-bearing sperm, whereas females produce only X-bearing eggs. Therefore, the difference between male and female gametes is the sex chromosome. Throughout evolution, sex chromosomes have become the driving force for sexual development and differentiation, brain development, and reproduction. Emerging evidence from the recent sex chromosome sequencing projects indicates that both X and Y chromosomes harbor large amounts of X- and/or Y-specific repetitive sequences originated mainly from autosome–to–sex chromosome transposition/retroposition events. Through the transposition/retroposition system, sex chromosomes frequently exchange genetic materials with autosomes in a lineage-specific manner and accumulate a disproportionate amount of reproduction-related genes, expressed predominantly in the testis. These testis genes are usually amplified in the X- and/or Y-specific repetitive sequences (i.e., ampliconic) and are difficult to sequence and to be properly annotated. Furthermore, the nearly identical multicopy nature of the testis gene families on the sex chromosomes and their broad functions in immunity (such as CTAs in cancer development) and brain function

complicate research work on their molecular mechanisms. For those reasons, the roles of all sex chromosome–linked CTA/CTX gene families in reproduction are poorly understood. Future research should proceed in three general directions. First, researchers should seek to annotate sex chromosome–linked reproduction-related genes in as many mammalian species as possible, with an aim to building a complete list of sex chromosome–linked reproductive genes. This will lead to the discovery of a core set of genes important for reproduction in mammals. Second, researchers should dissect the molecular function of each member in a given testis gene family. Efforts should be made to generate animal models by either the routine gene-knockout approach or new technologies such as CRISPR/Cas 9. Finally, researchers should study the impact of lost Y chromosome lineages on a population (such as has been done in dairy cattle) regarding reproduction and survivability. The effective population size of dairy cattle has been extremely reduced in the past 50 years as a consequence of artificial selection and the application of artificial insemination technology (29, 172). This problem may occur in other livestock species in which artificial insemination has been applied. It is my belief that the more we study the sex chromosomes, the closer we come to understanding evolution, speciation, and reproduction in mammals.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Chad Dechow and Dr. Cooduvalli Shashikant for careful reading of the manuscript and for their helpful comments and suggestions. This work was supported, in part, by the National Institute of Food and Agriculture, US Department of Agriculture (grant no. 2018-67015-27576), and by a TSF-CURE grant from the Pennsylvania Department of Health to W.-S. Liu.

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