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# Functional significance of the sex chromosomes during spermatogenesis

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## Abstract

Mammalian sex chromosomes arose from an ordinary pair of autosomes. Over hundreds of millions of years, they have evolved into highly divergent X and Y chromosomes and have become increasingly specialized for male reproduction. Both sex chromosomes have acquired and amplified testis-specific genes, suggestive of roles in spermatogenesis. To understand how the sex chromosome genes participate in the regulation of spermatogenesis, we review genes, including single-copy, multi-copy, and ampliconic genes, whose spermatogenic functions have been demonstrated in mouse genetic studies. Sex chromosomes are subject to chromosome-wide transcriptional silencing in meiotic and postmeiotic stages of spermatogenesis. We also discuss particular sex-linked genes that escape postmeiotic silencing and their evolutionary implications. The unique gene contents and genomic structures of the sex chromosomes reflect their strategies to express genes at various stages of spermatogenesis and reveal the driving forces that shape their evolution.

## Introduction

Mammalian sex chromosomes arose from an ordinary pair of autosomes about 200–300 million years ago (Bellott, et al. 2014, Lahn and Page 1999, Ohno 1967, Ross, et al. 2005). Since then, the X has preserved most of the ancestral autosomal genes, while the Y has lost most of them and only kept a selective group of critical genes (Bellott, et al. 2014, Hughes, et al. 2012, Hughes, et al. 2010, Ross, et al. 2005, Skaletsky, et al. 2003, Soh, et al. 2014). Remarkably, both chromosomes have acquired a substantial amount of ampliconic sequences, from which amplified genes are expressed predominantly in the testicular germ cells (Bellott, et al. 2010, Mueller, et al. 2013, Soh, et al. 2014). Several ancestral genes on the human and mouse Y chromosomes are also found to undergo amplification and change their broad expression pattern to a testis-specific one (Bellott, et al. 2014, Skaletsky, et al.

2003, Soh, et al. 2014). These findings suggest an evolutionary trend of increasing specialization for male reproduction for the sex chromosomes.

Male reproduction relies on functional spermatogenesis, which consists of cells of four major differentiation stages: spermatogonia (mitotic), spermatocyte (meiotic), spermatid (postmeiotic; spermiogenic), and spermatozoa (sperm) (Figure 1). Spermatogenesis genes are abundant in sex chromosomes. Based on the genomic structures, these genes can be divided into two major groups: single-copy genes and ampliconic/multi-copy genes. The majority of single-copy genes involved in spermatogenesis are ancestral genes and tend to be broadly expressed in the body (examples in Table 1). There are also a small group of single-copy genes that are specifically expressed in the spermatogenic cells, some of which have been shown to exert critical functions in spermatogenesis (Wang, et al. 2001, Zheng, et al. 2010). In contrast, most of the ampliconic/multi-copy genes were acquired following the divergence of the X and Y chromosomes and are expressed predominantly in the male germline. Interestingly, a few newly acquired genes on the sex chromosomes that have not been amplified, for instance, *Prssly* and *Teyorf1* on the mouse Y chromosome (Soh, et al. 2014), also show a germline-specific expression pattern.

Spermatogenic cells undergo meiosis to generate haploid gametes (Figure. 1). Unlike autosomes that undergo synapsis along their entire lengths during meiosis, large regions of the X and Y chromosomes remain unsynapsed and trigger transcriptional silencing, called meiotic sex chromosome inactivation (MSCI: reviewed elsewhere (Ichijima, et al. 2012, Turner 2007, van der Heijden, et al. 2011)). MSCI is accompanied by the formation of distinct heterochromatin called the XY body (also known as the sex body). This chromosome-wide silencing is maintained into round spermatids by postmeiotic sex chromatin (PMSC) (Greaves, et al. 2006, Namekawa, et al. 2006, Turner, et al. 2006). Importantly, there is a group of sex-linked genes that escape postmeiotic silencing and become expressed in postmeiotic spermatids (reviewed elsewhere (Sin and Namekawa 2013)). The regulatory mechanisms by which sex-linked genes are inactivated by MSCI and activated to escape postmeiotic silencing were identified as DNA damage response pathways adopted from somatic machinery to recognize damaged DNA (Broering, et al. 2014, Ichijima, et al. 2011, Sin, et al. 2012a, Turner, et al. 2004). The genes that escape postmeiotic silencing, termed escape genes, include most of the ampliconic/multi-copy genes, as well as many single-copy genes, on both X and Y chromosomes (Cocquet, et al. 2009, Mueller, et al. 2008, Sin, et al. 2012b, Toure, et al. 2004a). Therefore, the unique gene contents and genomic structures of the sex chromosomes reflect their strategies to express genes during critical stages of spermatogenesis. In this review article, we summarize the genomic structure of the sex chromosomes and the functions of single-copy and ampliconic/ multi-copy genes in the regulation of spermatogenesis, and try to shed light on the evolution of their unique genomic properties.

## Genomic structure of the sex chromosomes

The differentiation of the sex chromosomes from ancestral autosomes was initiated when one of the autosome pairs acquired a sex-determining gene and underwent chromosomal inversion that prevented recombination in the region (Bellott, et al. 2014, Lahn and Page

1999, Ohno 1967). Since then, the X and Y chromosomes have evolved in their own way (described below), but share a striking common feature: gaining specialization for male reproduction through acquisition and amplification of testis-specific genes (Bellott, et al. 2014, Bellott, et al. 2010, Hughes, et al. 2012, Hughes, et al. 2010, Mueller, et al. 2008, Mueller, et al. 2013, Ross, et al. 2005, Skaletsky, et al. 2003, Soh, et al. 2014, Warburton, et al. 2004). The recent improvement in the accuracy of sex chromosome sequence assemblies, by means of the Single-Haplotype Iterative Mapping and Sequencing (SHIMS) technique, has allowed for detailed annotation of large palindromic and/or ampliconic sequences and for comparisons of sex chromosomes among species (Bellott, et al. 2014, Bellott, et al. 2010, Hughes, et al. 2012, Hughes, et al. 2010, Mueller, et al. 2013, Skaletsky, et al. 2003, Soh, et al. 2014).

## X chromosome

The present-day human X chromosome has preserved 98% of genes from the ancestral autosome; however, due to an intergenic expansion of non-coding sequences (particularly long interspersed repeat elements and retroviral sequences) during evolution, the gene density on the X chromosome is about half of the average of all autosomes (Bailey, et al. 2000, Bellott, et al. 2010, Lander, et al. 2001, Ross, et al. 2005). Despite the low gene density, the X chromosome has acquired a substantial number of protein-coding genes, the majority of which are members of multi-copy gene families, including cancer/testis antigen genes, and exhibit testis-predominant expression patterns (Bellott, et al. 2010, Mueller, et al. 2013, Scanlan, et al. 2004). These features are also seen in the sex chromosomes of other mammals, such as chimpanzees, rhesus monkeys and mice (Soh, et al. 2014). In the avian ZW system, the Z chromosome that is shared by both sexes (equivalent to the X chromosome in the XY system, though males are the homogametic sex) also has a lower gene density than the autosomes due to increased intergenic distances, and contains a massive tandem amplification of genes that are expressed in the testis (Bellott, et al. 2010). Thus, the convergent acquisition and amplification of testis-expressed genes biases the X, as well as Z, chromosomes toward male reproduction functions.

## Y chromosome

The human Y chromosome retains only 3% of its ancestral genes, due to a lack of a crossover partner in the male-specific region, leading to genetic decay (Bellott, et al. 2010, Charlesworth and Charlesworth 2000, Skaletsky, et al. 2003). Since the start of its differentiation, the human Y chromosome has undergone evolutionary decay at least four times in a step-wise fashion, as evidenced by the presence of the "evolutionary strata" of the X-Y pairs of ancestral genes (Lahn and Page 1999). After each event that created a stratum (*e.g.*, chromosomal inversions), genes in the newly non-recombining region of the Y chromosome decayed rapidly at first, followed by a stable phase, leaving a constant set of surviving genes (Hughes, et al. 2012). These surviving ancestral genes were selected during evolution for their critical functions in males: male sex determination, sperm production, and viability (Bellott, et al. 2014). In addition to surviving ancestral genes, the Y chromosome acquired and amplified genes that are predominantly expressed in the testis (Hughes, et al. 2012, Hughes, et al. 2010, Skaletsky, et al. 2003, Soh, et al. 2014). Interestingly, ancestral genes on the human Y chromosome, such as *Rbmy*, *Tspy*, and *Hsfy*,

evolved into multi-copy and testis-specific genes, in contrast to other Y-linked ancestral genes, which are broadly expressed (Bellott, et al. 2014). Two ancestral genes on the mouse Y chromosome, *Rbmy* and *Zfy*, have also undergone amplification and turned into germ cell-specific genes (Mahadevaiah, et al. 1998, Mardon, et al. 1989, Soh, et al. 2014, Vernet, et al. 2014).

### Lineage-specific acquisition and amplification

The amplified testis-specific genes on the X and Y chromosomes are rapidly evolving and demonstrate relatively recent and lineage-specific acquisition (Hughes, et al. 2010, Mueller, et al. 2013, Soh, et al. 2014). The recent improvement in the accuracy of the human and mouse sex chromosome sequence assemblies allows for a detailed comparison of the ampliconic regions between the two species (Mueller, et al. 2013, Soh, et al. 2014). Different from the single-copy genes that are highly conserved among placental mammals, most of the ampliconic genes are independently acquired and amplified between the human and mouse lineages (Mueller, et al. 2013, Soh, et al. 2014). Similarly, a comparison of the Y chromosome sequence between human and a closely related species, chimpanzee, also suggests a substantial change in the gene content and genomic structures in the ampliconic regions (Hughes, et al. 2010). Therefore, both X and Y chromosomes are subject to selective evolutionary forces that increase specialization for lineage-specific male reproductive functions.

## Functions of single-copy genes vs. ampliconic/multi-copy genes during spermatogenesis

Spermatogenesis is a biological process where self-renewing mitotic spermatogonia give rise to mature spermatozoa through two major differentiation steps: meiosis and spermiogenesis. Spermatogenic cells reside in the testis, which provides a somatic environment to support spermatogenesis. Two major somatic cell types are Sertoli cells (which nourish germ cells through stages of spermatogenesis within the seminiferous tubule) and Leydig cells (which produce and regulate hormones, such as androgens). According to currently available mouse genetic studies, genes on the sex chromosomes regulate spermatogenesis in many different ways (Table 1). Most X-linked single-copy genes that exhibit function in spermatogenesis are expressed in multiple tissues and control the process through either somatic and/or germ cells, but a small group of spermatogonially expressed, germ cell-specific genes have also been identified (Wang, et al. 2001). With the exception of the male sex-determining gene, *Sry*, surviving ancestral Y-linked single-copy genes are broadly expressed, both in the adult and throughout development (Bellott, et al. 2014). In contrast, ampliconic/multi-copy genes on the sex chromosomes tend to be expressed specifically in male germ cells, with potential roles in postmeiotic spermatids (Cocquet, et al. 2010, Comptour, et al. 2014, Mueller, et al. 2008, Mueller, et al. 2013, Reynard, et al. 2009, Riel, et al. 2013, Sin, et al. 2012b). In this section, we provide examples of single-copy and ampliconic/multi-copy genes from mouse genetic studies in the regulation of spermatogenesis.

## X-linked spermatogonial genes

A systematic genomic screen for spermatogonially expressed, germ cell-specific genes in mice has identified 36 genes, 11 of which are on the X chromosome and 3 of which are on the Y chromosome (Wang, et al. 2001). Further genetic studies on 4 out of the 11 X-linked genes (*Tex11*, *Taf7l*, *Nxf2*, *Tktl1*) in mice have been reported. *Tex11* is critical for meiotic prophase by promoting synapsis and crossover formation in spermatocytes. Deletion of *Tex11* spanning from exon 3 to exon 29 leads to apoptosis of spermatocytes at the pachytene stage due to meiotic failure (Yang, et al. 2008). However, a different *Tex11* mutant mouse line, with a deletion at only exon 3, leads to a premature termination of translation and exhibits milder meiotic defects and normal fertility (Adelman and Petrini 2008). *Taf7l* encodes a germ cell-specific subunit of the TFIID complex that is essential for polymerase II-mediated transcription (Cheng, et al. 2007, Pointud, et al. 2003). Although *Taf7l* is expressed in spermatogonia, spermatocytes, and spermatids, its targeted deletion causes a relatively mild phenotype associated with a reduction in sperm counts and sperm motility. The mild phenotype may be due to a functional compensation by its ubiquitously expressed autosomal paralog, *Taf7*, which was retrotransposed from an mRNA of the *Taf7l* gene (Cheng, et al. 2007, Pointud, et al. 2003). Interestingly, *Tex11 Taf7l* double knockout mice show a much more severe meiotic phenotype than either single mutant: an earlier spermatocyte death at the zygotene stage, suggesting a synergistic regulation of both genes in meiotic prophase (Zheng, et al. 2010). *Nxf2,* a nuclear mRNA export factor, has implicated roles in mRNA stability and trafficking (Lai, et al. 2006, Takano, et al. 2007, Tretyakova, et al. 2005). Male mice lacking *Nxf2* show reduced fertility, resulting from defects in spermatogonial proliferation and meiotic chromosome segregation (Pan, et al. 2009). In contrast to the other 3 genes, targeted deletion of *Tktl1* in mice did not yield any obvious phenotype in reproduction (Bentz, et al. 2011).

*Scml2*, another critical X-linked gene in spermatogenesis was recently identified in proteomics screens. SCML2, a germline-specific Polycomb protein, was identified as a component of γH2AX (histone variant H2AX phosphorylated at serine 139, an essential histone modification of the XY body)-containing nucleosomes from testes (Hasegawa, et al. 2015). Another proteomics study independently identified SCML2 as a meiosis-specific protein (Luo, et al. 2013). *Scml2* is highly transcribed in spermatogonia, but then subject to MSCI in spermatocytes due to its X-linkage (Hasegawa, et al. 2015), consistent with a previous report using RNA FISH (fluorescence *in situ* hybridization) analysis (Mueller, et al. 2008). SCML2 protein is detected in the entire nuclei of undifferentiated spermatogonia and persists until meiosis, where it becomes accumulated on the XY body despite its transcriptional silencing (Hasegawa, et al. 2015, Luo, et al. 2015). *Scml2* knockout mice are infertile (Hasegawa, et al. 2015), while an independent *Scml2* knockout mouse line with a distinct genetic background exhibits severely compromised fertility (Luo, et al. 2015). SCML2 has two distinct functions between autosomes and sex chromosomes in the establishment of the epigenome during spermatogenesis. On autosomes in spermatogonia, SCML2 positively regulates mono-ubiquitinated histone H2A at lysine 119 (H2AK119ub) and, during spermatogenic differentiation, suppresses genes commonly expressed among somatic cells as well as spermatogenesis-progenitor genes (Hasegawa, et al. 2015). Paradoxically, on sex chromosomes during meiosis, SCML2 prevents H2AK119ub

(Hasegawa, et al. 2015, Luo, et al. 2015), thereby enabling unique epigenetic programming of sex chromosomes for male reproduction. Therefore, the X chromosome carries the critical regulator SCML2 for its own gene regulation during spermatogenesis—as is the case for the X-linked *Xist* non-coding RNA, and the proteins RLIM and ATRX in the regulation of female X chromosome inactivation (Lee and Bartolomei 2013, Sarma, et al. 2014, Shin, et al. 2010).

## Ancestral single-copy and multi-copy genes

Male reproduction relies on successful male sex differentiation and development of an individual. Two major genes, *Sry* and *Ar*, which drive male differentiation, are located on the Y and X chromosomes, respectively (Gubbay, et al. 1990, Sinclair, et al. 1990, Wang, et al. 2009). *Sry* initiates testis differentiation of the bipotential gonad in mid-gestation (Koopman, et al. 1991a). The testis then secretes androgens whose functions are mediated through an androgen receptor, encoded from *Ar*, to trigger the differentiation of male sex organs (*e.g.*, external genitalia and internal accessory organs) and secondary characteristics (Wang, et al. 2009). While androgen receptor signaling in germ cells is not required for spermatogenesis, androgen receptor signaling in the surrounding Sertoli, Leydig, and peritubular myoid cells plays critical roles in the completion of sperm production (Chang, et al. 2004, De Gendt, et al. 2004, Holdcraft and Braun 2004, Lyon and Hawkes 1970, Xu, et al. 2007, Yeh, et al. 2002, Zhang, et al. 2006). Other X- and Y-linked genes that have been shown to regulate spermatogenesis, based on mouse genetic studies, are listed in Table 1. In particular, targeted deletion of either *Sox3* or *Tsc22d3* impairs spermatogonial differentiation (Bruscoli, et al. 2012, Laronda and Jameson 2011). Similar to the function of the spermatogonia-specific gene *Nxf2*, disruption of *Pcyt1b* causes an age-dependent depletion of spermatogenic cells, indicating a role in the maintenance of spermatogonial stem cells (Jackowski, et al. 2004).

The function of Y-linked genes has been mainly studied in mice carrying spontaneous deletions (see review in (Burgoyne 1998)), due to the difficulty of generating targeted mutations on the Y chromosome using conventional homologous recombination methods in embryonic stem (ES) cells. Nevertheless, these studies have revealed the critical functions of the Y chromosome in spermatogenesis. The Y chromosome is the only chromosome that is acrocentric in the mouse genome, while all others are telocentric (Mouse Genome Sequencing, et al. 2002, Soh, et al. 2014). Its long arm contains the highly amplified germ cell-specific gene families *Sly*, *Srsy*, and *Ssty*, all of which were acquired during the evolution of the rodent lineage (Soh, et al. 2014). Mice lacking part or all of the long-arm genes show mild-to-severe defects in sperm morphology, fertilization, and fertility (Burgoyne, et al. 1992, Conway, et al. 1994, Riel, et al. 2013, Suh, et al. 1989, Toure, et al. 2004b). On the short arm, *Eif2s3y* is the sole factor essential for spermatogonial proliferation (Mazeyrat, et al. 2001). In mice lacking the entire Y chromosome (XO), introduction of two transgenes expressing *Sry* and *Eif2s3y* are sufficient to initiate testis differentiation and drive spermatogenesis through meiosis up to the round spermatid stage (Yamauchi, et al. 2014). Using similar transgene rescue strategies to study ancestral multi-copy genes on the mouse Y chromosome, *Zfy2* is found to play an important function in meiotic checkpoints that remove spermatocytes with synaptic errors (Royo, et al. 2010). *Zfy2,* together with *Zfy1* and

*Zfx* (an X homolog), has been discovered to exert a major role in promoting the completion of the 2$^{nd}$ division of meiosis (Vernet, et al. 2011, Vernet, et al. 2014).

### Acquired ampliconic/multi-copy genes that escape postmeiotic silencing

During meiosis in spermatocytes, MSCI results in an almost complete shut-down of sex-linked genes, besides a few exceptions, such as the non-coding RNA *Tsx* (Anguera, et al. 2011) and some microRNA genes (Song, et al. 2009). On the other hand, many more sex-linked genes escape from postmeiotic silencing in round spermatids. Based on microarray analysis, 13% of mouse X-linked genes escape postmeiotic silencing while 87% remain repressed in round spermatids (Namekawa, et al. 2006). Subsequent studies demonstrated that most ampliconic/multi-copy genes on the mouse X chromosome (33 gene families, representing ~273 genes in ampliconic regions and non-ampliconic regions) are highly expressed in the round spermatids (Mueller, et al. 2008, Sin, et al. 2012b). Because conventional targeting knockout strategies cannot be applied to multicopy genes, their function has been investigated through the generation of transgenic mice carrying small interfering RNAs. For instance, using shRNAs to knock down *Slx* (~25 copies), *Slxl1* (~14 copies), or both, mice showed impairments in sperm motility and counts (Cocquet, et al. 2010). While *Slx* or *Slxl1* knockdown mice were subfertile, double *Slx/Slxl1* transgenic mice were sterile due to more apparent defects in spermatid elongation and sperm release (Cocquet, et al. 2010). These data suggest that X-linked ampliconic/multi-copy genes escape postmeiotic silencing for a functional role in spermiogenesis.

Additionally, Y-linked ampliconic/multi-copy genes are also known to escape postmeiotic silencing (Toure, et al. 2005). For instance, *Sly* (126 copies) is located on the long arm of the Y chromosome and expressed in postmeiotic spermatids (Reynard, et al. 2009, Soh, et al. 2014). Using a shRNA transgene to knock down *Sly*, mice exhibited sperm head abnormalities and reduced fertility (Cocquet, et al. 2009). In addition, knockdown of *Sly* also leads to sperm DNA damage and defective chromatin packaging (Riel, et al. 2013). Interestingly, SLY protein accumulates on PMSC, and *Sly* knockdown causes derepression of sex-linked genes in spermatids, suggesting that *Sly* escapes postmeiotic silencing to regulate PMSC (Cocquet, et al. 2009).

### Single-copy genes that escape postmeiotic silencing

In addition to ampliconic/multi-copy genes, there are also many single-copy genes that escape postmeiotic silencing. One example of a single-copy escape gene from mouse genetic studies is *Akap4,* a gene critical for sperm motility function by encoding a protein anchoring a critical enzyme, cyclic AMP-dependent protein kinase, in the fibrous sheath of the sperm flagellum (Carrera, et al. 1994, Miki, et al. 2002b). *Akap4* belongs to a group of genes that commonly escape postmeiotic silencing in both human and mouse, and is also involved in sperm function in humans (Luconi, et al. 2011). Expectedly, deletion of *Akap4* in male mice leads to infertility due to abnormal sperm morphology and motility (Miki, et al. 2002a). On the other hand, not all of the escape genes are essential for fertility. *Rlim* (also known as *Rnf12*) escapes postmeiotic silencing in mice; however, *Rlim* is not required for male fertility, although maternal RLIM is a critical regulator of imprinted X chromosome inactivation in embryos (Shin, et al. 2010).

### Human sex-linked genes that escape postmeiotic silencing

In humans, although the involvement of the X chromosome in male fertility is in debate (Stouffs, et al. 2009), the Y chromosome microdeletions clearly contribute to spermatogenic defects (Krausz, et al. 2011, Stouffs and Lissens 2012). Escape genes in humans have also been implicated in male infertility. In addition to *AKAP4*, the X-linked single-copy escape gene *TEX13A* may be associated with azoospermia and abnormal sperm maturation (Lee, et al. 2003, Luconi, et al. 2011). Mutations of other X-linked escape genes, such as *CUL4B* and *AFF2*, are associated with hypergonadism and developmental delay, suggesting a possible function in male reproduction (Isidor, et al. 2010, Sahoo, et al. 2011). Further investigation of escape genes is warranted for a better understanding of human male infertility.

## Evolutionary implications of genes that escape postmeiotic silencing

Recent advances in the biology of sex chromosomes, summarized above, enable us to revisit old evolutionary theories of sex chromosomes. In this section, we discuss the driving forces and strategies that have shaped the evolution of the sex chromosomes. MSCI is common between eutherians and marsupials (Hornecker, et al. 2007, Namekawa, et al. 2007) and suggested to contribute to the emergence of the X and Y chromosomes in therian ancestors (Potrzebowski, et al. 2008). With this history, gene reactivation within the context of sex chromosome inactivation was a necessary step to acquire the spermiogenesis functions of the sex chromosomes.

The mammalian X chromosome is enriched with male reproductive genes (Sin, et al. 2012b, Wang, et al. 2001, Zhang, et al. 2010). It has been shown that these male-biased genes tend to be those recently acquired on the X chromosome and expressed in round spermatids (Zhang, et al. 2010). Acquired genes include both single-copy and ampliconic/multi-copy genes. While genes subject to postmeiotic silencing are highly conserved between humans and mice, male reproductive genes that escape postmeiotic silencing are significantly diverged (Sin, et al. 2012b). Compared with non-escape genes, escape genes exhibit higher rates of amino acid changes calculated by Ka/Ks values (Ka/Ks value: the ratio of the number of non-synonymous substitutions per non-synonymous site ($K_a$) to the number of synonymous substitutions per synonymous site ($K_s$)). In addition, although these escape genes are considered important to sperm function, different species tend to have different sets of escape genes (Sin, et al. 2012b). In the study looking at X-linked escape genes, of 54 mouse and 66 human genes, only 12 of them were common between the two species. For example, *Rlim* escapes postmeiotic silencing in mice but is subject to postmeiotic silencing in humans (Sin, et al. 2012b). It remains unknown how the expression change of *Rlim* between mice and humans affects reproductive fitness. Such expression change in the round spermatids was also observed between two closely related mouse species, suggesting that the expression of escape genes could also be an evolutionary constraint, separating the species (Homolka, et al. 2011). Furthermore, 25 out of 54 mouse escape gene are rodent-specific, and 8 out of 66 human escape genes are primate/ape-specific. Such specificities suggest that escape genes evolved rapidly in both sequence and expression pattern, and are likely to contribute to reproductive isolation between species (Sin, et al. 2012b).

### Rice's hypothesis

These findings are in accord with long-standing evolutionary theories of sex chromosomes. Due to sexual antagonisms (*i.e.*, conflicting fitness between males and females during sexual reproduction), the sex chromosomes accumulated sexually antagonistic alleles that are favored in one sex but detrimental to the other (Rice 1984). Rice hypothesized that the X chromosome is enriched for genes benefiting males due to the selection of hemizygously expressed favorable effects in males, while their deleterious effects in females would initially be hidden by heterozygosity (Rice 1984). Similarly, any recessive mutation that is advantageous for male fitness can more likely spread on the X chromosome rather than an autosome, where the effects are masked by heterozygosity and likely to be lost. The Y chromosome is also predicted to be enriched for male-beneficial genes because of the hemizygous expression of Y-linked genes and selection that only occurs in males.

### Ohno's law

A subsequent study compared the sequences of human and mouse X chromosomes and showed that species-specific sequences apparently reside in the ampliconic regions, suggesting that these genes are significantly diverged and are, for the most part, independently acquired between these two species (Mueller, et al. 2013). This study challenged Ohno's law (Ohno 1967), stating that the gene contents of the X chromosome is conserved across placental mammals due to the somatic dosage compensation between the X chromosome and autosomes (*i.e.*, any translocations between the X chromosome and autosomes would disturb the gene dosage). Genes that are expressed before meiosis and subject to postmeiotic silencing are found to be highly conserved (Sin, et al. 2012b) and thus follow Ohno's law. However, ampliconic genes that are predominantly expressed in postmeiotic spermatids escape not only postmeiotic silencing, but also Ohno's law. Therefore, Ohno's law pertains mainly to the genes subject to postmeiotic silencing, whereas sexual antagonism underlies escape gene activation in postmeiotic spermatids, consistent with Rice's hypothesis (Figure 2). These findings suggest that sex chromosomes face at least two antagonistic evolutionary driving forces: (1) sex chromosome inactivation, which preserves the gene content (mostly ancestral genes) among species; and (2) sexual antagonism, which facilitates escape gene activation and the acquisition of new genes.

### Intragenomic conflict between X and Y chromosomes

The human and mouse sex chromosomes contain X-Y pairs of ampliconic gene families that were co-acquired and amplified during their evolution (Skaletsky, et al. 2003, Soh, et al. 2014). Due to the high divergence in sequence between these pairs of gene families, it has been thought that they may have resulted from intragenomic conflict, which may affect transmission disorders (or segregation distortion) by the action of selfish genetic elements transmitted to the next generation at a frequency higher than the expected Mendelian inheritance ratio. This hypothesis has been tested in mice by knocking down the ampliconic gene family pair: for instance, *Slx/Slxl1* on the X chromosome and *Sly* on the Y chromosome (Cocquet, et al. 2012). *Slx/Slxl1* deficiency leads to male-biased litters, and *Sly* deficiency leads to female-biased litters (Cocquet, et al. 2009, Cocquet, et al. 2010). However, double knockdown of *Slx/Slxl1* and *Sly* rescues the sex ratio distortion phenotype and the fertility

problem caused by either knockdown (Cocquet, et al. 2012). Based on this result, it was proposed that SLX/SLXL1 and SLY are responsible for intragenomic conflict between X - and Y-bearing sperm by differentially regulating sperm function (Cocquet, et al. 2012, Good 2012). Further, Y-linked SSTY also localizes on PMSC, interacts with SLX/SLX1 and SLY, and is proposed to be involved in the intragenomic conflict (Comptour, et al. 2014). Similar to *Slx/Slxl1-Sly*, other acquired ampliconic gene family pairs on the mouse sex chromosomes, including *Sstx-Ssty1/Ssty2* and *Srsx-Srsy*, are rodent-specific and show significant divergence in copy number and sequence (Soh, et al. 2014). Taken together, the intragenomic conflict has an important impact on the evolution of sex chromosomes by co-acquiring and amplifying genes that escape postmeiotic silencing and regulate spermatid differentiation, which is in line with Rice's hypothesis. Given that these genes are rapidly evolving in a lineage-specific manner, they may also contribute to the speciation process.

## Concluding remark

Mammalian sex chromosomes possess unique genomic features and gene expression that might have conferred an advantage in reproductive fitness of the species. Sequence analysis has revealed that both the X and Y chromosomes contain testis-specific ampliconic/multi-copy gene families, most of which were acquired during recent evolution, and some of which were derived from ancestral single-copy genes. Many of these genes escape postmeiotic silencing, suggesting that they exert important functions in spermatid differentiation and maturation. Because they are rapidly evolving and are highly divergent in gene content among species, it is thought that they contribute to the speciation process. Similar to ampliconic/multi-copy genes, single-copy escape genes are found to evolve rapidly in sequence and vary markedly between human and mouse (Sin, et al. 2012b). Therefore, the accumulation of ampliconic/multi-copy genes and genes escaping postmeiotic silencing on the sex chromosomes is likely to benefit male reproduction in a lineage-specific manner, thus supporting Rice's hypothesis (Figure 2). In contrast, genes that are subject to postmeiotic silencing tend to be more conserved and thus follow Ohno's law (Figure 2). Therefore, these antagonistic driving forces and strategies have shaped the evolution of the sex chromosomes.

## Looking forward

In spite of accumulating evidence, the mechanisms that drive unique genomic arrangements and expression, such as amplification of genomic elements and escape from postmeiotic silencing, remain unknown. A promising path for future investigation is to identify the potential mechanisms that induce gene amplification and regulate gene expression during the postmeiotic period of spermatogenesis. Curiously, ampliconic DNA regions exhibit unique epigenetic signatures, such as DNA hypomethylation, throughout the germline prior to gene activation (Ikeda, et al. 2013). It suggests that the uniquely evolved ampliconic sequences are distinctly regulated during spermatogenesis.

Furthermore, in humans, sex chromosome abnormalities such as Kleinfelter (XXY) and Turner (XO) syndromes are associated with infertility, suggesting that the gene dosage of the sex chromosomes is also critical for fertility both in males and females (Heard and

Turner 2011). Thus, in addition to the function of individual genes, the chromosome-wide regulation of gene dosage of the sex chromosomes may be warranted for further investigation.

Another interesting direction for future investigation is the relationship between the function of sex-chromosome genes and some types of cancer. Large palindromic and/or repetitive sequences of the sex chromosomes are enriched with cancer/testis (CT) antigens that are commonly expressed in testes and cancer (Simpson, et al. 2005). Unique genomic and epigenomic features likely underlie germline-specific gene activation on the sex chromosomes, as well as ectopic expression in some types of cancers. It would be intriguing to investigate how the germline program recapitulates in the case of abnormal somatic cells such as cancer cells, and how to safeguard and maintain our genome through the germline. The extraordinary genomic structure and genetic complement of sex chromosomes are likely to be key players in such processes.

## Acknowledgments

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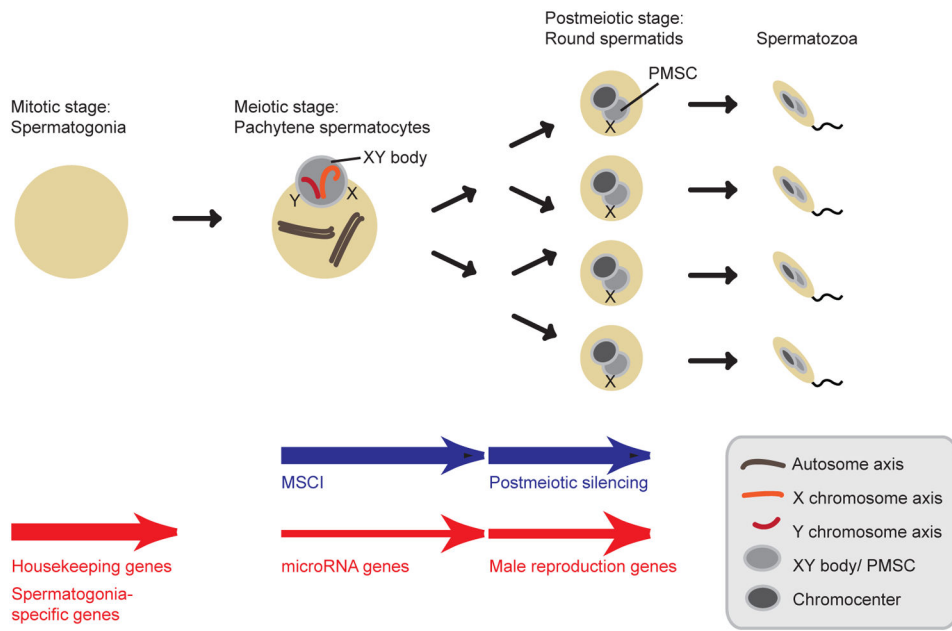
**Figure 1. Schematic of spermatogenesis and events on the sex chromosomes**

Due to sex chromosome inactivation, sex chromosomes function in two critical phases before meiosis in mitotically proliferating spermatogonia and after meiosis in haploid spermatids. Blue arrows: two stages of sex chromosome inactivation, Red arrows: Groups of genes expressed from the sex chromosomes.
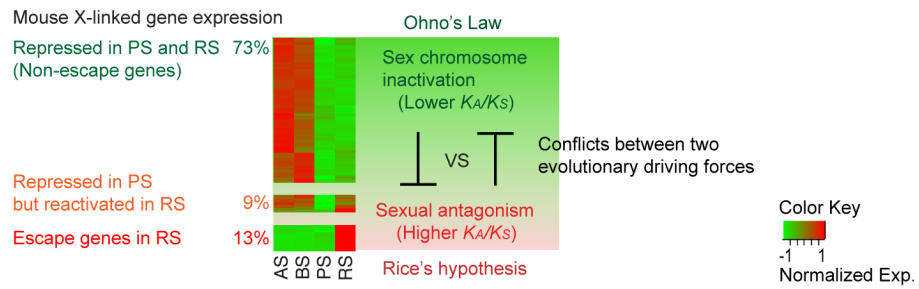
**Figure 2. Model of conflict between two evolutionary driving forces on the X chromosome**
Microarray heatmap adapted from Namekawa *et al.* 2006. AS: type A spermatogonia, BS:
type B spermatogonia, PS: pachytene spermatocytes, RS: round spermatids.

**Table 1**

Sex-chromosome genes shown to contribute to male fertility in mouse genetic studies

| Gene name | Biological function | Expression | Human homolog on the X; associated diseases | Fertility phenotype of genetically altered XY mice | References |
|---|---|---|---|---|---|
| X-linked genes | | | | | |
| Abcd1 | Peroxisomal transporter of very long-chain fatty acids | Multiple tissues | Yes; Adrenoleukodystrophy | Reduced fertility; increased number of interstitial cells with accumulated lipid inclusions | (Forss-Petter, et al. 1997) |
| Akap4 | A-kinase anchoring protein | Spermatids, spermatozoa | Yes | Infertility; abnormal sperm flagellum morphology and motility | (Miki, et al. 2002a) |
| Ar | Androgen receptor | Multiple tissues | Yes; Androgen Insensitivity syndrome | Infertility; testicular feminization; impaired spermatogenesis; tissue-specific KOs indicate that AR signaling in Sertoli, Leydig, and peritubular myoid cells is crucial for spermatogenesis | (Chang, et al. 2004, De Gendt, et al. 2004, Holdcraft and Braun 2004, Lyon and Hawkes 1970, Xu, et al. 2007, Yeh, et al. 2002, Zhang, et al. 2006) |
| Arx | Homeodomain transcription factor | Multiple tissues | Yes; Early-onset epileptic encephalopathy 1 and Partington syndrome | Small testis; fetal Leydig cell differentiation defect | (Kitamura, et al. 2002, Miyabayashi, et al. 2013) |
| Atp7a | Copper transpoting ATPase | Multiple tissues | Yes; Menkes disease | Infertility; spermatogenic cell death; poor sperm morphology and motility | (Kotula-Balak, et al. 2007, Kowal, et al. 2010, Llanos, et al. 2006) |
| Cdk16 | Cyclin-dependent kinase | Multiple tissues | Yes | Infertility; abnormal sperm morphology and motility | (Mikolcevic, et al. 2012) |
| Dmd | Dystrophin | Multiple tissues | Yes; Duchenne and Becker muscular dystrophy | Reduced fertility; abnormal sperm flagella morphology and motility | (Hernandez-Gonzalez, et al. 2005, Kudoh, et al. 2005) |
| Fmr1 | Fragile X mental retardation protein | Multiple tissues | Yes; Fragile X syndrome | Macroorchidism-associated increase in Sertoli cell proliferation; defective synapsis and crossover formation in spermatocytes | (Alpatov, et al. 2014, Slegtenhorst-Eegdeman, et al. 1998) |
| Foxp3 | Forkhead transcription factor | Multiple | Yes; Immunodysregulation, polyendocrinopathy, enteropathy | Infertility; hypogonadism and arrested spermatogenesis caused by insufficient pituitary gonadotropins | (Jasurda, et al. 2014) |
| Gpr64 | G protein-coupled receptor | Proximal epididymis, efferent ductules | Yes | Age-dependent infertility; accumulation of spermatozoa in the efferent ductules caused by defective fluid reabsorption | (Davies, et al. 2004) |
| Gria3 | Ionotropic glutamate receptor | Multiple tissues | Yes; Mental retardation X-linked 94 | Reduced fertility with unknown causes | (Meng, et al. 2003) |
| L1cam | Neural cell adhesion molecule | Multiple tissues | Yes; Hydrocephalus, CRASH and MASA syndromes | Infertility at high frequency with unknown causes | (Cohen, et al. 1998) |

| Gene name | Biological function | Expression | Human homolog on the X; associated diseases | Fertility phenotype of genetically altered XY mice | References |
|---|---|---|---|---|---|
| Mecp2 | Methyl CpG binding protein | Multiple tissues | Yes; Rett Syndrome | Infertility; cryptorchidism | (Guy, et al. 2001) |
| NrOb1 | Nuclear receptor | Multiple tissues | Yes; 46XY sex reversal; Adrenal hypoplasia | Infertility; impaired spermatogenesis; multiple defects in Sertoli, Leydig, and peritubular myoid cells | (Jeffs, et al. 2001, Meeks, et al. 2003, Yu, et al. 1998) |
| Nxf2 | Nuclear RNA export factor | Spermatogonia | Yes | Reduced fertility; age-dependent depletion of spermatogonial cells; meiotic arrest; reduced sperm count and motility | (Pan, et al. 2009) |
| Pcyt1b | CTP: phosphocholine cytidylyltransferase | Multiple tissues | Yes | Reduced fertility; age-dependent depletion of spermatogonial cells | (Jackowski, et al. 2004) |
| Porcn | Membrane-bound O- acyl transferase | Multiple tissues | Yes; Focal dermal hypoplasia | Small testis; abnormal vas deferens morphology | (Liu, et al. 2012) |
| Prdx4 | Peroxiredoxin | Multiple tissues | Yes | Reduced testis weight; increased spermatogenic cell death by oxidative damage; reduced sperm counts | (Iuchi, et al. 2009) |
| Rhox5 | Reproductive homeodomain transcription factor | Multiple tissues | Yes[*] | Reduced fertility caused by defective Sertoli cells; increased spermatocyte death; reduced sperm counts and motility | (Maclean, et al. 2005) |
| Scml2 | Epigenetic regulator, Polycomb protein | Spermatogenic cells | Yes; Medulloblastomas | Infertility; suppression of somatic/ progenitor genes during spermatogenesis; suppression of histone H2A ubiquitination on the sex chromosomes | (Hasegawa, et al. 2015, Luo, et al. 2015) |
| Slx/Slxl-1 | Sycp3-like X-linked | Spermatogenic cells | No | Reduced fertility in single knockdowns; infertility in double knockdowns; impaired spermiogenesis leading to cell death; abnormal sperm morphology; reduced sperm counts and motility[**] | (Cocquet, et al. 2012, Cocquet, et al. 2010) |
| Sms | Spermine synthase | Multiple tissues | Yes; Snyder-Robinson syndrome | Infertility with unknown causes | (White, et al. 2013) |
| Sox3 | High-mobility group box transcription factor | Multiple tissues | Yes; Mental retardation and panhypopituitarism | Reduced testis weight; spermatogonial differentiation defects; reduced sperm counts | (Laronda and Jameson 2011, Rizzoti, et al. 2004, Weiss, et al. 2003) |
| Taf7l | TATA box binding protein (TBP)- associated factor | Spermatogenic cells | Yes | Reduced fertility; reduced sperm counts; abnormal sperm morphology and motility | (Cheng, et al. 2007) |
| Tex11 | Meiotic protein | Spermatogenic cells | Yes | Depletion of spermatocytes caused by meiotic defects | (Adelman and Petrini 2008, Yang, et al. 2008) |

| Gene name | Biological function | Expression | Human homolog on the X; associated diseases | Fertility phenotype of genetically altered XY mice | References |
|---|---|---|---|---|---|
| *Tsc22d3* | Glucocorticoid- induced leucine zipper protein | Spermatogenic cells | Yes | Infertility; defects in proliferation and differentiation of undifferentiated spermatogonia; massive cell death during meiotic prophase | (Bruscoli, et al. 2012) |
| *Tsx* | Non-coding RNA | Multiple tissues | No | Grossly undisturbed fertility, albeit smaller testis and partial germ cell death during meiotic prophase | (Anguera, et al. 2011) |
| *Zfx* | Zinc finger protein | Multiple tissues | Yes | Grossly undisturbed fertility, albeit smaller testis and reduced sperm counts | (Luoh, et al. 1997) |
| Y-linked genes | | | | | |
| *Sry* | High-mobility group box transcription factor | Fetal gonad and brain | Yes; 46XY sex reversal | Infertility; male-to-female sex reversal | (Koopman, et al. 1991b, Lovell-Badge and Robertson 1990) |
| *Eif2s3y* | Translation initiation factor | Multiple tissues | No | Essential for spermatogonial proliferation [**] | (Mazeyrat, et al. 2001) |
| *Sly* | Sycp3-like Y-linked | Spermatogenic cells | No | Reduced fertility; impaired spermiogenesis; sperm DNA damage; abnormal sperm chromatin packaging and morphology | (Cocquet, et al. 2012, Cocquet, et al. 2009, Riel, et al. 2013) |
| *Zfy1* | Zinc finger transcription factor | Spermatogenic cells | Yes | Functions in promoting 2nd meiotic divisions in spermatocytes [***] | (Vernet, et al. 2011, Vernet, et al. 2014) |
| *Zfy2* | Zinc finger transcription factor | Spermatogenic cells | Yes | Functions in meiotic checkpoints and divisions in spermatocytes [***] | (Vernet, et al. 2011, Vernet, et al. 2014) |

[*] Gene belongs to a Rhox homeobox gene cluster. Due to a rapid evolution of this gene cluster, a human homolog cannot be precisely defined.

[**] Genes belong to multi-copy gene families. The phenotypes were demonstrated in male transgenic mice carrying specific siRNA transgenes.

[***] Gene function was demonstrated by transgene rescue experiments in male mice lacking various portions of the Y chromosome.