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.

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Introduction

Mammalian sex chromosomes arose from an ordinary pair of autosomes ~200–300 million years ago (Ohno 1967, Lahn & Page 1999, Bellott et al. 2014). 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 (Skaletsky et al. 2003, Bellott et al. 2010, 2014, Hughes et al. 2010, 2012, Mueller et al. 2013, 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, 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), spermatocytes (meiotic), spermatids (postmeiotic; spermiogenic), and spermatozoa (sperm) (Fig. 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 they 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, Prsly 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 (Fig. 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 previously (Turner 2007, van der Heijden et al. 2011, Ichijima et al. 2012)). 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 previously Sin & 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 (Turner et al. 2004, Ichijima et al. 2011, Sin et al. 2012a, Broering et al. 2014). 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 (Toure et al. 2004a, Cocquet et al. 2009). 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 shed light on the evolution of their unique genomic properties.

Genomic structure of the sex chromosomes


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, Lander et al. 2001, Ross et al. 2005, Bellott et al. 2010). 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 (Scanlan et al. 2004, Bellott et al. 2010, Mueller et al. 2013). These features are also observed 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 (Skaletsky et al. 2003, Bellott et al. 2010). Since the start of its differentiation, the human Y chromosome has undergone evolutionary decay at least four times in a step-wise manner, as evidenced by the presence of the ‘evolutionary strata’ of the X–Y pairs of ancestral genes (Lahn & 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 (Skaletsky et al. 2003, Hughes et al. 2010, 2012, 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 (Mardon et al. 1989, Mahadevaiah et al. 1998, 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 somatic and/or germ cells, but a small group of spermatagonially 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 (Mueller et al. 2008, 2013, Reynard et al. 2009, Cocquet et al. 2010, Riel et al. 2013, Comptour et al. 2014). 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 three of which are on the Y chromosome (Wang et al. 2001). Further genetic studies on four out of the 11 X-linked genes (Tex11, Tafl7l, Nxf2, and Tktl1) in mice have been reported. Tex11 is critical for meiotic prophase by promoting synopsis 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
# Table 1 Sex-chromosome genes shown to contribute to male fertility in mouse genetic studies.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Biological function</th>
<th>Expression</th>
<th>Human homolog on the X; associated diseases</th>
<th>Fertility phenotype of genetically altered XY mice</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-linked genes</td>
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</tr>
<tr>
<td>Abcd1</td>
<td>Peroxisomal transporter of very long-chain fatty acids</td>
<td>Multiple tissues</td>
<td>Yes; adrenoleukodystrophy</td>
<td>Reduced fertility; increased number of interstitial cells with accumulated lipid inclusions</td>
<td>Forss-Petter et al. (1997)</td>
</tr>
<tr>
<td>Akap4</td>
<td>A-kinase anchoring protein</td>
<td>Spermatids, spermatozoa</td>
<td>Yes</td>
<td>Infertility; abnormal sperm flagellum morphology and motility</td>
<td>Miki et al. (2002)</td>
</tr>
<tr>
<td>Ar</td>
<td>Androgen receptor</td>
<td>Multiple tissues</td>
<td>Yes; androgen insensitivity syndrome</td>
<td>Infertility; testicular feminization; impaired spermatogenesis; tissue-specific KOs indicate that AR signaling in Sertoli, Leydig, and peritubular myoid cells is crucial for spermatogenesis</td>
<td>Lyon &amp; Hawkes (1970), Yeh et al. (2002), Chang et al. (2004), De Gendt et al. (2004), Zhang et al. (2006) and Xu et al. (2007)</td>
</tr>
<tr>
<td>Arx</td>
<td>Homeodomain transcription factor</td>
<td>Multiple tissues</td>
<td>Yes; early-onset epileptic encephalopathy 1 and Partington syndrome</td>
<td>Small testis; fetal Leydig cell differentiation defect</td>
<td>Kitamura et al. (2002) and Miyabayashi et al. (2013)</td>
</tr>
<tr>
<td>Atp7a</td>
<td>Copper transpotting ATPase</td>
<td>Multiple tissues</td>
<td>Yes; Menkes disease</td>
<td>Infertility; spermatogenic cell death; poor sperm morphology and motility</td>
<td>Llanos et al. (2006), Kotula-Balak et al. (2007) and Kowal et al. (2010)</td>
</tr>
<tr>
<td>Cdk16</td>
<td>Cyclin-dependent kinase</td>
<td>Multiple tissues</td>
<td>Yes</td>
<td>Infertility; abnormal sperm morphology and motility</td>
<td>Mikolcevic et al. (2012)</td>
</tr>
<tr>
<td>Dmd</td>
<td>Dystrophin</td>
<td>Multiple tissues</td>
<td>Yes; Duchenne and Becker muscular dystrophy</td>
<td>Reduced fertility; abnormal sperm flagellum morphology and motility</td>
<td>Hernandez-Gonzalez et al. (2005) and Kudoh et al. (2005)</td>
</tr>
<tr>
<td>Fmr1</td>
<td>Fragile X mental retardation protein</td>
<td>Multiple tissues</td>
<td>Yes; Fragile X syndrome</td>
<td>Macroorchidism-associated increase in Sertoli cell proliferation; defective synapsis and crossover in spermatocytes</td>
<td>Slegtenhorst-Eegdeman et al. (1998) and Alpatov et al. (2014)</td>
</tr>
<tr>
<td>Foxp3</td>
<td>Forkhead transcription factor</td>
<td>Multiple</td>
<td>Yes; immunodysregulation, polyendocrinopathy, enteropathy</td>
<td>Infertility; hypogonadism and arrested spermatogenesis caused by insufficient pituitary gonadotropins</td>
<td>Jasurda et al. (2014)</td>
</tr>
<tr>
<td>Gpr64</td>
<td>G protein-coupled receptor</td>
<td>Proximal epididymis, efferent ductules</td>
<td>Yes</td>
<td>Age-dependent infertility; accumulation of spermatozoa in the efferent ductules caused by defective fluid reabsorption</td>
<td>Davies et al. (2004)</td>
</tr>
<tr>
<td>Gria3</td>
<td>Ionotropic glutamate receptor</td>
<td>Multiple tissues</td>
<td>Yes; mental retardation X-linked 94</td>
<td>Reduced fertility with unknown causes</td>
<td>Meng et al. (2003)</td>
</tr>
<tr>
<td>L1cam</td>
<td>Neural cell adhesion molecule</td>
<td>Multiple tissues</td>
<td>Yes; hydrocephalus, CRASH and MASA syndromes</td>
<td>Infertility at high frequency with unknown causes</td>
<td>Cohen et al. (1998)</td>
</tr>
<tr>
<td>Mecp2</td>
<td>Methyl CpG-binding protein</td>
<td>Multiple tissues</td>
<td>Yes; Rett syndrome</td>
<td>Infertility; cryptorchidism</td>
<td>Guy et al. (2001)</td>
</tr>
<tr>
<td>Nr0b1</td>
<td>Nuclear receptor</td>
<td>Multiple tissues</td>
<td>Yes; 46XY sex reversal; adrenal hypoplasia</td>
<td>Infertility; impaired spermatogenesis; multiple defects in Sertoli, Leydig, and peritubular myoid cells</td>
<td>Yu et al. (1998), Jeffs et al. (2001) and Meeks et al. (2003)</td>
</tr>
<tr>
<td>Nxd2</td>
<td>Nuclear RNA export factor</td>
<td>Spermatogonia</td>
<td>Yes</td>
<td>Reduced fertility; age-dependent depletion of spermatogonial cells; meiotic arrest; reduced sperm count and motility</td>
<td>Pan et al. (2009)</td>
</tr>
<tr>
<td>Pcyt1b</td>
<td>CTP: phosphocholine cytidylyltransferase</td>
<td>Multiple tissues</td>
<td>Yes</td>
<td>Reduced fertility; age-dependent depletion of spermatogonial cells</td>
<td>Jackowski et al. (2004)</td>
</tr>
<tr>
<td>Porcn</td>
<td>Membrane-bound O-acyltransferase</td>
<td>Multiple tissues</td>
<td>Yes; focal dermal hypoplasia</td>
<td>Small testis; abnormal vas deferens morphology</td>
<td>Liu et al. (2012)</td>
</tr>
<tr>
<td>Prdx4</td>
<td>Peroxiredoxin</td>
<td>Multiple tissues</td>
<td>Yes</td>
<td>Reduced testis weight; increased spermatogenic cell death by oxidative damage; reduced sperm counts</td>
<td>Iuchi et al. (2009)</td>
</tr>
<tr>
<td>RhoX5</td>
<td>Reproductive homeodomain transcription factor</td>
<td>Multiple tissues</td>
<td>Yes</td>
<td>Reduced fertility caused by defective Sertoli cells; increased spermatocyte death; reduced sperm counts and motility</td>
<td>Maclean et al. (2005)</td>
</tr>
</tbody>
</table>

References:
- Llanos et al. (2006), Kotula-Balak et al. (2007) and Kowal et al. (2010)
- Mikolcevic et al. (2012)
- Hernandez-Gonzalez et al. (2005) and Kudoh et al. (2005)
- Slegtenhorst-Eegdeman et al. (1998) and Alpatov et al. (2014)
- Jasurda et al. (2014)
- Davies et al. (2004)
- Meng et al. (2003)
- Cohen et al. (1998)
- Guy et al. (2001)
- Yu et al. (1998), Jeffs et al. (2001) and Meeks et al. (2003)
- Pan et al. (2009)
- Jackowski et al. (2004)
- Liu et al. (2012)
- Iuchi et al. (2009)
- Maclean et al. (2005)
deletion at only exon 3, leads to a premature termination of translation and exhibits milder meiotic defects and normal fertility (Adelman & Petrini 2008). *Taf7l* encodes a germ cell-specific subunit of the TFIIID complex that is essential for polymerase II-mediated transcription (Pointud et al. 2003, Cheng et al. 2007). 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 (Pointud et al. 2003, Cheng et al. 2007). Interestingly, *Tex11 Taf7l* double-knockout mice show a much more severe meiotic phenotype than either single mutant: an earlier knockout mice show a much more severe meiotic

### Table 1 Continued.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Biological function</th>
<th>Expression</th>
<th>Human homolog on the X; associated diseases</th>
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</tr>
</thead>
<tbody>
<tr>
<td><em>Scm12</em></td>
<td>Epi- and Polycomb regulator</td>
<td>Spermatogenic cells</td>
<td>Yes; Medulloblastomas</td>
<td>Infertility; suppression of somatic/progenitor genes during spermatogenesis; suppression of histone H2A ubiquitination on the sex chromosomes</td>
<td>Hasegawa et al. (2015) and Luo et al. (2015)</td>
</tr>
<tr>
<td><em>Sly</em></td>
<td>Zinc finger</td>
<td>Spermatogenic cells</td>
<td>Yes</td>
<td>Functions in meiotic checkpoints and divisions in spermatocytes</td>
<td>Vernet et al. (2011, 2014)</td>
</tr>
<tr>
<td><em>Sry</em></td>
<td>High-mobility group box transcription factor</td>
<td>Fetal gonad and brain</td>
<td>Yes; 46XY sex reversal</td>
<td>Infertility; male-to-female sex reversal</td>
<td>Lovell-Badge &amp; Robertson (1990) and Koopman et al. (1991)</td>
</tr>
<tr>
<td><em>Eif2s3y</em></td>
<td>Translation initiation factor</td>
<td>Multiple tissues</td>
<td>No</td>
<td>Essential for spermatogonial proliferation</td>
<td>Mazeiyrat et al. (2001)</td>
</tr>
<tr>
<td><em>Sly</em></td>
<td>Sycp3-like Y-linked</td>
<td>Spermatogenic cells</td>
<td>No</td>
<td>Reduced fertility; impaired spermiogenesis; sperm DNA damage; abnormal sperm chromatin packaging and morphology</td>
<td>Vernet et al. (2011, 2014)</td>
</tr>
<tr>
<td><em>Zfy1</em></td>
<td>Zinc finger transcription factor</td>
<td>Spermatogenic cells</td>
<td>Yes</td>
<td>Functions in promoting 2nd meiotic divisions in spermatocytes</td>
<td>Vernet et al. (2011, 2014)</td>
</tr>
<tr>
<td><em>Zfy2</em></td>
<td>Zinc finger transcription factor</td>
<td>Spermatogenic cells</td>
<td>Yes</td>
<td>Functions in meiotic checkpoints and divisions in spermatocytes</td>
<td>Vernet et al. (2011, 2014)</td>
</tr>
</tbody>
</table>

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

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

*c*Gene function was demonstrated by transgene rescue experiments in male mice lacking various portions of the Y chromosome.
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 its roles in mRNA stability and trafficking (Tretyakova et al. 2005, Lai et al. 2006, Takano et al. 2007). 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 three genes, targeted deletion of Tkt1 in mice did not yield any obvious phenotype in reproduction (Bentz et al. 2011).

Scml2, another critical X-linked gene in spermatogenesis, has been 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 transcriptional silencing (Hasegawa et al. 2015), SCML2 protein intensely accumulates in the entire nuclei of undifferentiated spermatogonia but disappears thereafter until the onset of meiosis, where it becomes accumulated on the XY body despite its disappearance (Hasegawa et al. 2015). SCML2 knockout mice are infertile (Hasegawa et al. 2015). 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 also 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 (Shin et al. 2010, Lee & Bartolomei 2013, Sarma et al. 2014).

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. 1991). 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 (Lyon & Hawkes 1970, Yeh et al. 2002, Chang et al. 2004, De Gendt et al. 2004, Holdcraft & Braun 2004, Zhang et al. 2006, Xu et al. 2007). 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 (Laronda & Jameson 2011, Bruscoli et al. 2012). Similar to the function of the spermatogonial-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 (for review, see 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 Consortium et al. 2002, Soh et al. 2014). Its long arm contains the highly amplified germ cell-specific gene families Sly, Sry, 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 (Suh et al. 1989, Burgoyne et al. 1992, Conway et al. 1994, Toure et al. 2004b). On the short arm, E112s3y 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 E112s3y is 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 2nd division of meiosis (Vernet et al. 2011, 2014).
Acquired ampliconic/multi-copy genes that escape postmeiotic silencing

During meiosis in spermatocytes, MSCI results in an almost complete shutdown of sex-linked genes, besides a few exceptions, such as the non-coding RNA Tlx (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). As conventional targeting knockdown strategies cannot be applied to multi-copy 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), Slx11 (14 copies), or both, mice showed impairments in sperm motility and counts (Mueller et al. 2008). While Slx or Slx11 knockdown mice were subfertile, double Slx/Slx11 knockdown 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 (Pour et al. 2005). For instance, Sly (126 copies) is located on the long arm of the Y chromosome and expressed in postmeiotic spermatids (Ichijima et al. 2011, 2012, Sin et al. 2012a, Sin & Namekawa 2013). Using an 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. 2002). 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. 2002). 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 (Cocquet et al. 2012, Good 2012).

Human sex-linked genes that escape postmeiotic silencing

In humans, although the involvement of the X chromosome in male fertility is a subject of debate (Stouffs et al. 2009, Stouffs & Lissens 2012), the Y chromosome microdeletions clearly contribute to spermatogenic defects (Krausz et al. 2011). 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, Hansen et al. 2010). 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 (Idior 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 is considered to have evolved with the emergence of the XY 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 (Wang et al. 2001, Zhang et al. 2010, Sin et al. 2012b). It has been shown that these male-biased genes tend to be those which have been 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 for 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 as to how the expression change in 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 genes are rodent specific, and eight out of 66 human escape genes are primate/ape specific. Such specificities suggest that escape genes evolved rapidly in both sequence and expression patterns and are likely to contribute to reproductive isolation between species (Sin et al. 2012b).

**Rice’s hypothesis**

These findings are in accordance with long-standing evolutionary theories of sex chromosomes. Owing 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 probably 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 are 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 (Fig. 2). These findings suggest that sex chromosomes face at least two antagonistic evolutionary driving forces: i) sex chromosome inactivation, which preserves the gene content (mostly ancestral genes) among species; and ii) 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). Owing 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

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**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.
intragenomic conflict (Comptour SLX1 and SLY, and is proposed to be involved in the Y-linked SSTY also localizes on PMSC, interacts with SLX/Slx/Slxl1 Sstx pairs on the mouse sex chromosomes, including sequence (Soh et al. 2014). Thus, intra-genomic 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. As 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 (Fig. 2). In contrast, genes that are subject to postmeiotic silencing tend to be more conserved and thus follow Ohno’s law (Fig. 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 before 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 Klinefelter (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 & 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 germ-line. The extraordinary genomic structure and genetic complement of sex chromosomes are likely to be key players in such processes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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