Trends in Genetics

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Opinion

Meiotic Executioner Genes Protect the Y from Extinction

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The Y has been described as a wimpy degraded relic of the X, with imminent demise should it lose sex-determining function. Why then has it persisted in almost all mammals? Here we present a novel mechanistic explanation for its evolutionary perseverance: the persistent Y hypothesis. The Y chromosome bears genes that act as their own judge, jury, and executioner in the tightly regulated meiotic surveillance pathways. These executioners are crucial for successful meiosis, yet need to be silenced during the meiotic sex chromosome inactivation window, otherwise germ cells die. Only rare transposition events to the X, where they remain subject to obligate meiotic silencing, are heritable, posing strong evolutionary constraint for the Y chromosome to persist.

Highlights

The mammalian Y chromosome has been proposed to be a wimp with impending demise should it lose sex-determining function.

Changes in sex-determining switches are common in non-mammalian vertebrates, so an alternative explanation is required to explain Y chromosome persistence.

The Y chromosome bears executioner genes that are essential for male meiosis, but must themselves also be subjected to meiotic silencing because they are pachytene lethal.

The only heritable location that executioner genes can transpose to is the X chromosome (just 5% of the genome), where they remain subject to appropriate meiotic silencing.

Transposition of executioner genes away from the Y chromosome is uncommon, posing strong evolutionary constraint for the Y to persistent.

Y Chromosome Evolution

Understanding the origin and fate of the Y chromosome in eukaryotes has been one of the most intriguing questions in biology for decades. After years of debate, the therian (marsupial and eutherian) mammal Y has been considered wimpy, fragile, and selfish, with its survival dependent on functions in **spermatogenesis** (see Glossary) and **sex determination** [1–3]. Yet, the Y chromosome has persisted, since its origin more than 165 million years ago (mya) [4], in all but a handful of mammalian species. This is in stark contrast to the high turnover of sex chromosomes in other vertebrate linages [5], begging for a mechanistic solution that can explain such persistence of the therian Y.

Most therian mammals have an XY male:XX female sex chromosome system, which evolved from a pair of autosomes [6,7] after the proto-Y chromosome acquired a dominant **testis-determining factor** (**TDF**, the *Sry* gene [8]). Male beneficial alleles then accumulated near this new TDF (in linkage disequilibrium), increasing their probability of being inherited in males. **Recombination** was ultimately suppressed across these alleles (possibly by inversion on the Y [9], although this is debated [10,11]), creating the first male-specific region of the Y, heralding the demise of non-dosage sensitive Y genes [12–14].

Without recombination, selection no longer acted on individual loci, rather the whole nonrecombining region of the Y. Throughout therian evolution, the Y lost functional genes and degraded, via **Muller's ratchet**, or selection at linked loci (**genetic hitchhiking/background selection**) [12]. Ultimately, only six ancient XY shared genes were retained in humans [13,14]. A further ten functional XY shared genes were retained from the autosomal region added to the sex chromosomes in the eutherian ancestor, but remain autosomal in marsupials [15,16]. By contrast to the Y, X chromosome gene order and content is generally well conserved in eutherians [17]. All that remains of once complete homology between the X and Y is a small **pseudoautosomal region (PAR)** (which varies between species) that synapses during male **meiosis** and within which there is an obligatory recombination event. The PAR is essential for proper sex chromosome pairing and segregation during meiosis, being one of the most recombinogenic regions of the genome [18–20].

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The loss of almost all functional genes provided inspiration for the wimpy Y hypothesis [1], which states that the Y is a degraded relic of the X. At a linear rate of decay, the Y could be lost in humans in approximately 10 million years [21]. However, Y gene loss has occurred in waves, with the current Y relatively stable and enriched for dosage-sensitive genes that might function in male viability, at least in humans [13]. The wimpy Y is complemented by the fragile Y hypothesis [2], which proposes that Ys with small PARs have a higher probability of erratic X and Y paring and segregation. The resultant aneuploidy stress imposes selective pressure to shift critical function from the Y to the autosomes. To reduce this aneuploidy stress, achiasmatic mechanisms (i.e., the **dense plate** in marsupials and synaptonemal complex structures in some rodent species) need to evolve to mediate faithful X and Y segregation [22–25]. Alternatively, an autosomal addition would be required to extend and rejuvenate the PAR [2].

PAR rejuvenation occurred in the eutherian ancestor under the addition-attrition model of sex chromosome evolution [26]. After PAR extension by an autosomal addition, recombination was further suppressed between the X and Y. The new male specific region of the Y was then subjected to the same degenerative forces as the original Y and suffered continued loss of gene function, wasting away. PAR rejuvenation is only a temporary escape from fragility, because as PAR size decreases the Y becomes increasingly fragile once more. Therefore, in the absence of cyclical PAR rejuvenation, or the evolution of an achiasmatic meiotic strategy, Y loss appears inescapable. In this wimpy and fragile scenario, the saving grace for the Y could be its selfish nature to recruit and retain genes from across the genome that have male beneficial function [27]. Once acquired, selection acted upon these new functions to retain the Y. In fact, the evolution of a new sex-determining switch (that replaces *Sry* function), and the removal of critical function in the testis, are generally considered the rare events that prevent Y loss [3]. So, the Y is wimpy in that it has lost almost all genes and in lineages with a small PAR it is fragile, but it is also selfish in recruiting male beneficial *genes.* However, is this air of selfishness enough to explain its long-term persistence in mammals?

Here we present the persistent Y hypothesis (Figure 1, Key Figure), which draws together intricacies of meiosis and sex chromosome evolution, providing a novel mechanistic explanation for perseverance of the eutherian mammal Y chromosome. Under persistent Y, the presence of Y-linked **meiotic executioner genes** [28,29] are a significant roadblock to Y loss. They are necessary for successful meiosis, but must also be subjected to **meiotic sex chromosome inactivation (MSCI)** [30–33]. If transposed to an autosome, ectopic expression during the meiotic silencing window is lethal to germ cells [28]. Because Y-to-autosome transpositions result in fatal meiotic arrest, they cannot be transmitted to the next generation, so the Y persists.

Unusual Sex Chromosome Systems

Despite the marsupial and eutherian sex chromosomes sharing a common origin more than 165 mya [4], the marsupial counterparts are smaller because they did not receive an autosomal addition (Figure 1). The marsupial X and Y do not share a PAR, so during prophase I of male meiosis, a dense plate structure, rich in synaptonemal complex proteins, has evolved to maintain XY pairing and avoid erratic X and Y segregation [24]. In northern brown bandicoot (*Isoodon macrourus*) the Y chromosome is eliminated from some somatic tissues [34,35], suggesting that it is losing function outside of the germline.

Eutherian mammals also display variant sex chromosome systems, which are especially evident in Muroidea rodents. These rodents usually have relatively small PARs, but in some species the PAR has been lost and achiasmatic meiotic strategies for XY pairing evolved as a result (e.g., gerbils [22], voles [36]). Atypical sex chromosome systems have also been reported. In

African pygmy mice (*Mus minutoides*) and two species of lemmings (*Myopus schisticolor* and *Dicrostonyx torquatus*), there are XX/ X*X/ X*Y females and XY males [37,38]. In creeping voles (*Microtus oregoni*) there are XY males and XO females [39], and in South American field mice (genus *Akodon*) XY females have arisen independently at least six times [40].

Despite examples of unusual eutherian sex chromosome systems, the Y chromosome persists, begging for a mechanism that can explain its presence. Species in which the Y has been lost are informative, but surprisingly rare. In mole voles, there were two independent losses of the Y [41], resulting in XX males and females (*Ellobius tancrei*, *Ellobius talpinus*, and *Ellobius alaicus*), and XO males and females (*Ellobius lutescens*) [37,42]. The Amami spiny rat (*Tokudaia osimensis*) has also suffered Y chromosome loss, with XO males and females [43]. In these species critical function has shifted from the Y and there is no functional copy of the testis-determining gene *Sry* (reviewed in [44]), so each must have evolved novel sex-determining switches and new sex chromosome systems.

Meiotic Silencing Forms Part of the Checkpoint Network

Differentiated sex chromosomes present challenges that need to be overcome in both the soma and germline. In somatic cells, sex chromosomes require **dosage compensation** to restore gene dose to original autosomal levels. In therian mammals, this involves complex interplay of upregulation of the single X in males and silencing of one X in the somatic cells of females (i.e., X chromosome inactivation) (reviewed in [45]). By contrast, it is during male meiosis (Box 1) when sex chromosomes (X and Y) are silenced, the MSCI phenomenon [30–33] (Box 2).

Ploidy reduction is essential for gametogenesis, so germ cells have developed a complex surveillance network. This includes three **meiotic checkpoints**: (i) response to unrepaired double-strand breaks (DSBs), (ii) transcriptional repression called **meiotic silencing of unsynapsed chromatin (MSUC)**, and (iii) the spindle assembly checkpoint (SAC) (reviewed in [46]) (Box 1). The first checkpoint comprises several layers of complexity, including: activation of DSB end-processing [47,48], suppression of inter-sister recombination [49,50], suppression of ectopic recombination [51], and the control of obligate crossover formation and crossover interference [52]. The second checkpoint detects the presence of partially or completely unsynapsed regions (e.g., extra chromosomes or chromosomal translocations), which, if triggered, induces MSUC [32,53,54], an epigenetic silencing program that is conserved across eukaryotes such as chicken (albeit transient [55]), *Drosophila* [56], grasshoppers [57], and *Caenorhabditis elegans* [58]. At metaphase I, the third checkpoint (SAC) assures proper segregation of homologous chromosomes (reviewed in [46]).

Executioner Genes Take Control over Spermatogenesis

Maintaining MSCI is essential for avoiding ectopic expression of male 'pachytene-lethal' genes (so-called 'executioner' genes [28,59]). This is true for the *Y*-linked *Zfy1* and *Zfy2* paralogs in the mouse, both encoding zinc finger transcription factors that, when ectopically expressed, induce pachytene arrest [46]. Although their molecular function remains elusive, transgenic mice have been informative in elucidating their role during spermatogenesis (Box 3).

In mice, there is a complex interplay between the expression/repression timing of executioner genes and the successful progression of meiosis through MSCI and the SAC [28,59]. *Zfy1*/2 are expressed in developing gonads and at specific stages of spermatogenesis in adult mice [60] (Box 1). During early prophase I, *Zfy1/2* expression triggers MSCI [59], but then must be subjected to the very silencing they induce. When inserted into an autosome as a transgene,

Glossary

Background selection: the reduction of genetic diversity at a nondeleterious locus because it is linked to a deleterious allele that is under negative selection.
Dense plate: a structure enriched in proteins of the synaptonemal complex formed during the first meiotic division of meiosis. It is necessary to maintain associated asynaptic and achiasmatic sex chromosomes that lack a PAR (e.g., in marsupials).
Dosage compensation: the process by which the single X in males and two Xs in females are balanced to have equivalent transcriptional output.
Genetic hitchhiking: the process by which a neutral allele achieves high frequency, or even fixation, within a population because it is linked to a beneficial allele that is under positive selection.
Meiotic executioner gene: a gene that if expressed during the MSCI window is lethal to the cell.
Meiosis: a conserved process in sexually reproducing organisms that results in the production of haploid gametes.
Meiotic checkpoints: wide range of interconnected molecular mechanisms that control chromosomal pairing, synapsis as well as the formation and repair of double-strand breaks during meiosis. It includes meiotic silencing of unsynapsed chromatin (MSUC) and the spindle assembly checkpoint (SAC).
Meiotic sex chromosome inactivation (MSCI): transcription silencing of the X and Y chromosomes during prophase I. The X and Y form a sex body (distinct from the autosomes) that is enriched for repressive histone modifications.
Meiotic silencing of unsynapsed chromatin (MSUC): the process by which unsynapsed chromosomal regions undergo a process of transcriptional inactivation during prophase I.
Muller's ratchet: in the absence of recombination, mutations on the Y chromosome cannot be removed from the population. As more mutations accumulate on the Y, the least-mutated Y is permanently lost. This is known as a click of the ratchet.
Post-meiotic sex chromatin (PMSC): a nuclear compartment, including the X and Y chromosomes, in post-meiotic spermatids, characterized by repressive histone modifications.

ectopic *Zfy1/2* expression through the MSCI window induces pachytene arrest, whereas prophase I progresses normally when inserted into the X as a transgene (and subject to MSCI). No other Y gene in mouse results in pachytene arrest when inappropriately expressed from an autosome [28] (Box 3).

Zfy1/2, along with their X-linked homolog (*Zfx*), also have roles at the SAC checkpoint and during spermiogenesis [29,61]. All three genes are expressed during interphase between the two meiotic divisions (i.e., leptotene/zygotene, interphasic secondary spermatocytes, and round spermatids) and play a regulatory role in their own silencing during MSCI [29]. All evidence suggest that *Zfy* genes are essential for MSCI, but when inappropriately expressed through the MSCI window are pachytene lethal, acting as their own sensor [29], so effectively their own judge, jury, and executioner.

Executioner Genes and a Persistent Y

With a tightly regulated surveillance system, meiosis poses strong evolutionary constraints on sex chromosome evolution. As outlined, executioner genes must be silenced during the MSCI window for meiosis to progress [28,59]. If translocated to an autosome they will be expressed ectopically and induce apoptosis (as evidenced in mouse models; Box 3). Therefore, the only genomic region executioner genes can be heritably translocated to is the X chromosome, reducing viable target sites to a maximum of approximately 5% of the genome (the typical size of the X in eutherian mammal Ensembl assemblies, although the mouse X is 8.8% of the genome). If relocated to the X, executioner genes must remain subject to obligate meiotic silencing and also retain their normal testis function, so that meiosis progresses. No other scenario will be tolerated by germ cells, making such movement away from the Y rare events.

Protection from Y chromosome loss is generally thought to result from maintenance of its functions in spermatogenesis and (more importantly) sex determination [3]. However, sex-determining switches are labile in non-mammalian vertebrates [5]. Additionally, movement of functional genes from the Y to autosomes is common in eutherian mammals [62], with only the transposition of non-executioner genes tolerated by germ cells. Here we propose that restricted movement of such executioner genes away from the Y (posed by their toxicity during MSCI) is the rate-limiting step to Y chromosome loss, explaining why such degraded and minimally functional Y chromosomes have persisted in almost all eutherian mammals. This would be true of any gene on the Y, with an essential function in meiosis, but proved to be detrimental when expressed during the MSCI window.

The question remains: what might these executioner elements be? In eutherian mammals, *Zfy* genes present themselves as a strong candidate from both functional (see earlier, all evidence supports that it must remain on the Y for correct meiotic progression) and evolutionary perspectives. *Zfy1/2* are among the five genes located on all eutherian Y chromosomes [13,14] and which have not been found retrotransposed or translocated to an autosome in any species [62]. There is also no evidence in public databases for paralogous *Zfy* duplication (except *Zfy1/2* in mouse). The other genes common to the Y in all eutherians are *Sry* (the testis-determining gene), along with the male fertility genes *Rbmy*, *Uty*, and *Ddx3y* [13,14]. Their expression during the MSCI window does not induce pachytene arrest [28], so they cannot act as executioner genes.

Species that have lost the Y provide further insight (e.g., *Ellobius* and *Tokudaia*). In *E. lutescens* and *T. osimensis* (both XO males and females), *Zfy* was independently translocated to the X [41,43], where it presumably remains subjected to MSCI, thus protecting from inappropriate

Pseudoautosomal region (PAR): homologous region on the X and Y chromosomes that pair during meiosis and within which there is an obligate recombination event. This is critical for proper segregation of the X and Y.
Recombination: the process by which homologous chromosomes exchange genetic material during the first meiotic division. It establishes physical connections between homologs that are essential for faithful chromosomal pairing and segregation.
Sex determination: the process by which an individual's phenotypic sex is determined. This can be controlled by a genetic switch or environmental cues (e.g., temperature). Sex determining switches are surprisingly varied in non-mammal vertebrates.
Spermatogenesis: the process by which male germ cells are formed. Spermatogenesis can be divided into three stages: (i) proliferation and differentiation of spermatogonia, (ii) meiosis, and (iii) spermiogenesis, the process of spermatid maturation to spermatozoa.
Testis-determining factor (TDF): a gene on the Y chromosome that triggers testis development. In therian mammals this is the *Sry* gene.

Key Figure

The Persistent Y Hypothesis

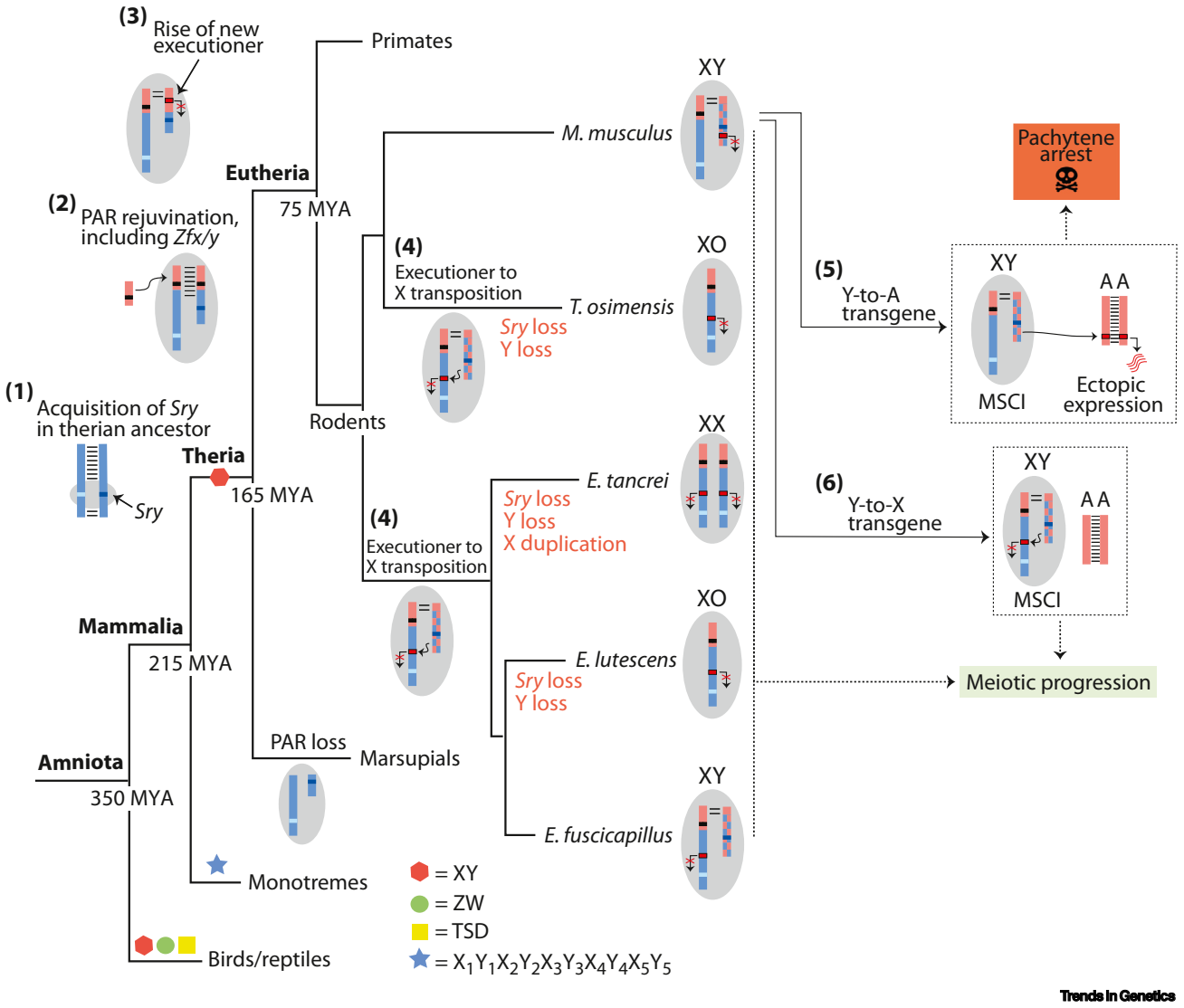

Figure 1. (1) The therian sex chromosomes arose with the acquisition of *Sry*, around which recombination was suppressed, and the Y began to degrade. Meiotic sex chromosome inactivation (MSCI) (grey shading) evolves as a result of the unpaired regions of the X and Y during prophase I. (2) The PAR was extended by the addition of an autosome in the eutherian ancestor, which included the executioner ancestor (*Zfx/y*). (3) After recombination was further suppressed, *Zfy* gains executioner function. Irrespective of further degradation and gene loss, the Y chromosome is now persistent as silencing of *Zfy* is essential for meiotic progression. The Y degrades and becomes a mosaic of conserved and added regions (checkered pattern). (4) The only heritable transposition of an executioner element away from the Y is to the X chromosome, where it remains subject to MSCI. Examples of such a transposition are provided by species from the genera *Ellobius* and *Tokudaia*, which subsequently suffered independent *Sry* loss and Y loss. (5) In mouse, ectopic executioner expression resulting from a Y-to-autosome transposition is pachytene lethal. (6) The same executioner transposed to the X remains subject to MSCI, so meiosis proceeds. Under the persistent Y-hypothesis, Y-linked executioner genes are necessary for MSCI initiation, but then must be subject to the very silencing they induce: they act as their own sensor. Only rare transposition events to the X chromosome (so silenced during MSCI) can be tolerated and passed to offspring. This protects the eutherian Y chromosome from loss. Branch lengths not to scale. Abbreviations: MYA, million years ago; TSD, temperature-dependent sex determination.

Box 1. Spermatogenesis Overview

Spermatogenesis is an intricate process resulting in the formation of haploid gametes (Figure I). This complex process is divided into three main stages: (i) proliferation and differentiation of spermatogonia; (ii) meiosis, two consecutive cell divisions (meiosis I and meiosis II) that produces haploid cells; and (iii) spermiogenesis. During the first meiotic prophase (prophase I) homologous chromosomes align, pair, synapse, and recombine, each interconnected through four sequential stages: leptotene, zygotene, pachytene, and diplotene [68]. At leptotene, chromatids start to condense and homologs begin to pair, a process that is facilitated by telomere clustering (the bouquet) at the nuclear envelope (reviewed in [69]). Homologous chromosomes initiate synapsis at zygotene, which along with recombination is completed at pachytene. At diplotene homologous chromosome segregate, which remain physically attached at sites where recombination took place [68]. The first meiotic division results in secondary spermatocytes, which undergo a short secondary meiotic division to produce round spermatids [68]. In mouse round spermatids sex chromosomes forms the **post-meiotic sex chromatin (PMSC)** [70]. Once meiosis is completed, spermatids undergo extensive chromatin remodeling during spermiogenesis, along with the morphological changes necessary for the formation of sperm [71].

Successful progression through meiosis is regulated by three meiotic checkpoints: (i) response to unrepaired DSBs, (ii) meiotic silencing of unsynapsed chromatin MSUC, and (iii) the spindle assembly checkpoint (SAC). The first two are regulated by the ATM/ATR pathway during prophase I and the SAC acts at metaphase I (reviewed in [27]). During prophase I, as homologous chromosomes pair, synapse, and recombine, there is tight monitoring of DSB formation and repair. As such, prophase I progression is dependent on faithful completion of two interconnected processes: the assembly of chromatin loops into chromosomal axes and the formation and repair of DSBs [72–74]. Given their paramount importance in regulating gametogenesis and despite differences in sensor and effector proteins, all three checkpoints are well conserved (reviewed in [27]).

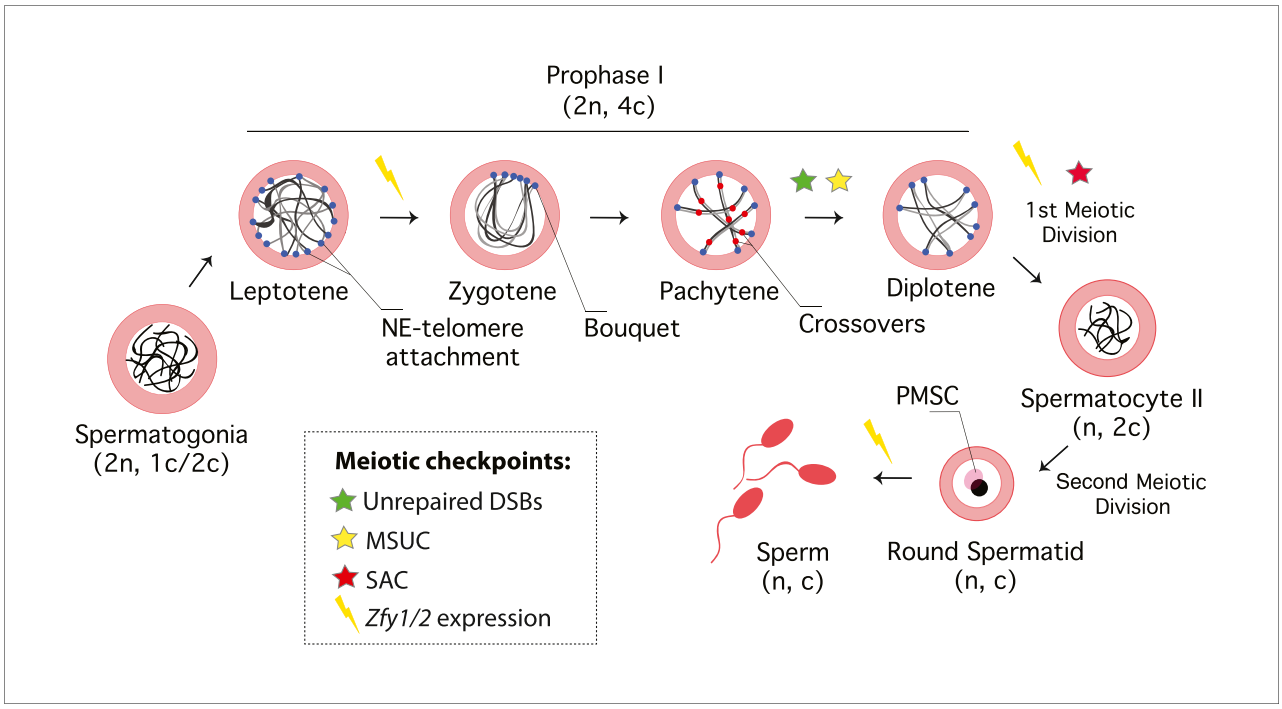

Figure I. Stages of Spermatogenesis. Abbreviations: DSB, double-strand break; MSUC, meiotic silencing of unsynapsed chromatin; NE, nuclear envelope; PMSC, post-meiotic sex chromatin; SAC, spindle assembly checkpoint. Adapted from [69].

expression during pachytene. In *E. talpinus* (XX males and females), *Zfy* was detected in the genome [41], but its genomic location has not been determined. For a gene that has persisted on the Y in all but one other eutherian genus, it is likely that *Zfy* was moved to the X in a single event, in which case it would also be located on the X in *E. talpinus*. Since *E. lutescens and Ellobius fuscocapillus* (XY male XX female) are sister taxa (Figure 1), *Zfy* would reside on the X in *E. fuscocapillus* too, although this has not been confirmed. In *E. talpinus* (with XX males and females) it might be expected that there would be no MSCI in males, on account of the pairing of X chromosomes. However, the two Xs only partially synapse and form a γH2AX-positive sex body [63], so *Zfy* is probably subject to appropriate silencing. Therefore, we

Box 2. How Are Sex Chromosomes Inactivated During Prophase I?

Sex chromosomes are subjected to silencing in male meiosis by a phenomenon called meiotic sex chromosome inactivation (MSCI), a sex chromosome-specific extension of MSUC (reviewed in [75]). This renders the genetically different X- and Y-bearing gametes functionally similar during the first meiotic division. Although initial observations in mouse suggested that MSCI would be restricted to the heterogametic sex in species with heteromorphic sex chromosomes [76,77], MSCI is still observed in male mammals that have lost the Y chromosome (i.e., XO and XX [63]) (see Figure 1 in main text).

MSCI is characterized by an accumulation of chromatin modifications as a response to asynapsed chromatin during prophase I [32,78]. DSBs generated genome-wide in leptotene are repaired by the DNA damage response machinery during zygotene via homology search. In the case of differentiated sex chromosomes (i.e., XY), large portions of the X and Y chromosomes remain asynapsed and DSBs markers accumulate along their axes (i.e., RPA, DMC1, and RAD51) [79,80]. The presence of such markers induces the ATM/ATR pathway to catalyze a first wave of organized histone H2AX phosphorylation (γH2AX) deposition as foci around DSBs [81–83]. Concurrently, asynapsed regions of the sex chromosomes are recognized by sensor proteins (such as CDK2, HORMAD1/2, and the BRCA1-A complex) that bind chromosomal axes (reviewed in [75]). These sensors trigger a second ATR-dependent (but independent of SPO11) wave of γH2AX that coats both the X and Y chromosomes, resulting in MSCI [32,79,80,84]. This creates a distinct sex chromosome-specific domain at pachytene (Figure I). The presence of γH2AX triggers the heterochromatization and silencing of the sex chromosomes, evidenced by the accumulation of histone marks such as H3K9me3/2, H2A ubiquitylation, and HP1β [54,70] and absence of active RNA polymerase II, H3K27m1/3, H3K9ac, and H4K16ac [70]. Although the molecular mechanisms that underpin this network are not completely understood, recent evidence suggests TRIM28 recruits SETDB1 (H3K9 methyltransferase) to the sex chromosomes to catalyze trimethylation of H3K9 [85]. The transcriptional silencing of MSCI is also coupled with 3D higher-order chromatin remodeling [86].

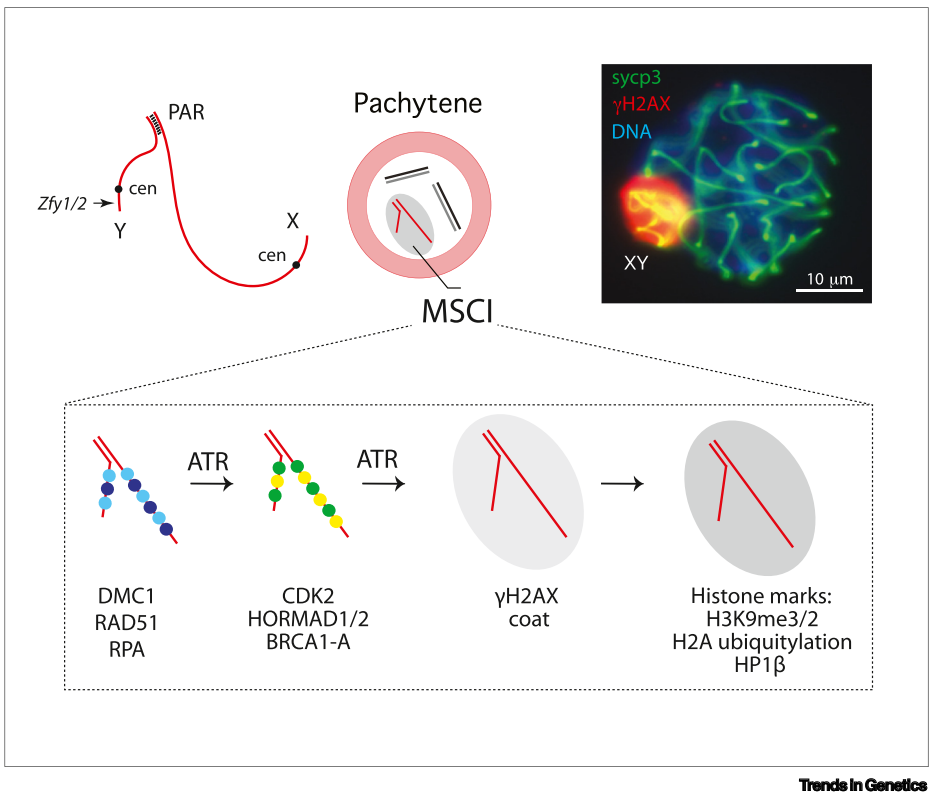

Figure I. Molecular Mechanisms of Meiotic Sex Chromosome Inactivation (MSCI).

speculate that *Zfy* transposition to the X in an *Ellobius* ancestor was the genetic predisposition required for Y loss.

Pigs with ectopic *Zfx/y* expression from an autosome during MSCI also suffer meiotic arrest [64], so it appears to act as an executioner gene even outside of rodents. However,

Box 3. Mouse Models Support *Zfy* Genes as Candidate Executioners

In XYY mice, the two Y chromosomes can fully synapse in primary spermatocytes (Figure I). As MSCI fails to form, this permits *Zfy1/2* ectopic expression resulting in pachytene arrest [28]. Although nine more Y-linked genes are expressed in XYY individuals during pachytene, only ectopic *Zfy1/2* expression results in pachytene arrest [28]. Expression of all other Y genes during pachytene from an autosomal transgene produces fertile males that pass on the transgene to offspring [28]. Importantly, when the *Zfy1/2* transgene is inserted into the X, it remains subject to MSCI, so meiosis progresses mirroring a wild type male phenotype.

Mice models with sex chromosome aneuploidies and different Y chromosome architecture also support an executioner role of *Zfy* genes. In $X^{Y}Y$ males (where a Y chromosome is fused to the X PAR), Y–Y pairing is prevented. Because the X^{Y} and Y do not pair, MSCI is activated (silencing the *Zfy* genes) and prophase I progresses [28]. The X chromosome in $XSxr^{b}O$ mice bears a part of Yp that includes a *Zfy1/2* fusion gene, *Sry* (resulting sex reversal), *H2al2y*, and *Rbmy* [87–89]. The *Zfy1/2* fusion gene consists of the last 6–11 exons of *Zfy1*, under the *Zfy2* promoter. In XO*Sry* mice, the only Y gene present is an autosomal *Sry* transgene [90]. In both $XSxr^{b}O$ and XO*Sry* primary spermatocytes progress through prophase I (MSCI is properly initiated, therefore silencing the *Zfy1/2* genes). However, the presence of a univalent X chromosome activates the spindle assembly checkpoint (SAC), resulting in meiotic failure [29,88] (Figure I). Despite this, a small proportion of cells escape apoptosis and continue through spermiogenesis, although with abnormal sperm, suggesting that *Zfy1/2* genes are important for sperm morphogenesis [91]. *Zfy1/2* genes along with *Zfx* (the related X-linked gene) are also involved in promoting meiosis II [29].

Collectively, these mouse models provide evidence for a complex role of *Zfy* genes during spermatogenesis. They are involved in four critical stages: initiation and progression through pachytene (their executioner role at the MSCI checkpoint), first meiotic metaphase (SAC), meiosis II, and sperm morphogenesis [28,29,59,88], with implications in fertilization and embryonic development [92]. The molecular mechanisms that underpin these functions remain unknown.

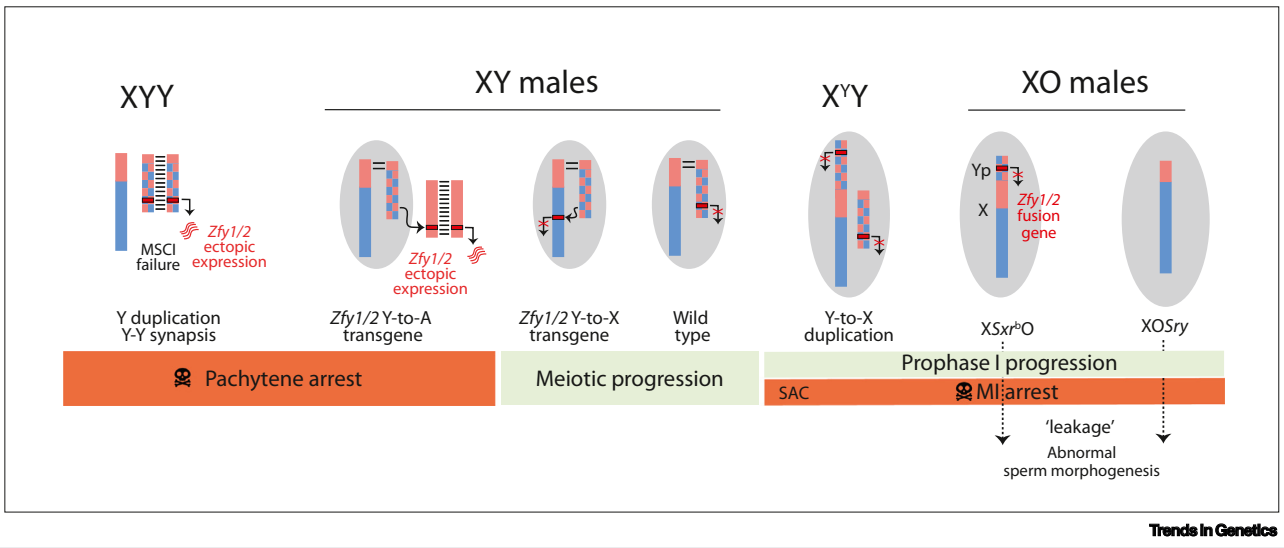

Figure I. Summary of *Zfy/x* Gene Mouse Models. Abbreviations: MI, Metaphase I; MSCI, meiotic sex chromosome inactivation; SAC, spindle assembly checkpoint.

Zfy can only be a eutherian-specific executioner, because in marsupials it remains on the autosome (in the same genomic context) [16] that was added to the X and Y in the eutherian ancestor. Sex chromosomes in marsupials are subjected to MSCI during prophase I, as in eutherians [65]. The marsupial sex body adopts a chromatin conformation during MSCI that extends to metaphase I, maintaining association of the X and Y chromosomes in the absence of physical synapsis [24,25]. This raises the possibility that there are yet to be discovered marsupial executioner genes that regulate sex chromosome silencing during prophase I, resulting in Y chromosome persistence. Ejection of the bandicoot Y chromosome from some somatic tissues [34,35] suggests that it is losing function, while Y maintenance in the germline could result from essential roles of executioner genes during meiosis. Alternatively, under the fragile Y hypothesis, species with an achiasmatic meiotic strategy (i.e., dense plate) have reduced aneuploid pressure due to faithful X and Y segregation. Reduced aneuploid stress would result in decreased pressure to remove critical function from the Y and subsequently less pressure for executioner genes to evolve that protect its demise.

Concluding Remarks

Although the eutherian Y is largely a fragile wimp, here we propose a new persistent Y scenario. It provides a long sought-after mechanistic solution to account for how such degraded and minimally functional Y chromosomes have endured, in all but a handful of mammalian species. The persistent Y adds new insight to a decades-old debate by drawing together meiotic functions of executioner genes with Y chromosome evolution. We outline how Y-linked genes act as their own judge, jury, and executioner during meiosis, as part of a tightly regulated germ cell surveillance system. They are crucial for successful meiosis, but must also be silenced during the MSCI window, otherwise they will execute the cell. This poses strong evolutionary constrains for the Y to persist, because only rare transposition events to the X (where they would be still subjected to obligate silencing during prophase I) can permit subsequent Y loss.

Such transpositions of *Zfy* to the X have happened in *Ellobius* and *Tokudaia* [41,43]. The transposition in *Ellobius* was likely a single event [41], so in *E. fuscocapillus*, *Zfy* is on the X chromosome, which means its Y chromosome lacks executioner protection and is in grave danger of loss. By contrast, *Zfy* was recently duplicated in mice [66], so two rare Y to X transposition events would be required to remove executioner protection. In this scenario, the mouse Y is extra persistent.

Under the persistent Y hypothesis, it is tempting to speculate whether executioner genes could be protecting Y chromosomes (and W chromosomes for that matter) in any linage with differentiated sex chromosomes that display MSCI (see Outstanding Questions). Are there linage-specific executioners, expression of which through a meiotic silencing window results in meiotic arrest? Understanding the intricacies of meiotic silencing in vertebrate and invertebrate species, with relatively long-lived (giving time for executioner function to evolve) heteromorphic sex chromosomes, will be particularly informative for driving our future understanding of sex chromosome evolution. Once proposed to be the candidate TDF [67], *Zfy* now presents itself as a protector of the Y in the form of meiotic executioner.

Outstanding Questions

How do executor genes originate? What are the evolutionary forces behind *Zfy* gaining its executor function? Is there prerequisite gene function for executor function to evolve?

What is the evolutionary origin and role of MSCI? How conserved is it in diverse vertebrate taxa? Does it provide the opportunity for meiotic executioner genes to evolve in many eukaryote linages?

Are different executioner genes present on the Y chromosome in marsupials and other vertebrate species? Or in any species with heteromorphic sex chromosomes and evidence of MSCI?

If executioner genes are common on Y chromosomes, are they functionally equivalent? Are they necessary for initiation of MSCI, and do they serve as their own sensor if expressed during the silencing window?

Is executioner gene function restricted to spermatogenesis (i.e., XY systems)? Or can executioners function from the W during oogenesis in female heterogametic (ZW) systems?

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