Sexual antagonism and X inactivation – the SAXI hypothesis

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X inactivation has evolved in the soma of mammalian females so that both sexes have the same ratio of X:autosomal gene expression. The X chromosome in the germ cells of XY males is also precociously inactivated for reasons that remain unclear. Unlike X inactivation in the soma, this germline X inactivation is not restricted to mammals but has evolved independently in several animal phyla. Thus, germline X inactivation might have been the precursor of somatic X inactivation in mammals. We now propose a hypothesis for the evolution of germline X inactivation. The hypothesis predicts a redistribution of late spermatogenic genes from the X chromosome to the autosomes, leading eventually to germline X inactivation as the X chromosome becomes 'demasculinized'. Sexual antagonism could be the mechanism driving this redistribution. Recent expression and genetic studies in mammals, nematodes and *Drosophila* support this hypothesis, and expression data on taxa that have not evolved germline X inactivation, such as birds and butterflies, should shed further light on it.

In mammals, one of the two copies of the X chromosome in the soma of females is inactivated to achieve the same ratio of X:autosomal gene expression as in males [1,2]. In a classic paper, Lifschytz and Lindsley [3] proposed that the X chromosome in the germ cells of XY males is also inactivated (earlier than the autosomes) and such precocious inactivation is necessary for completion of spermatogenesis. It has been suggested by many authors that the germline phenomenon could be the evolutionary precursor of the somatic event [2,4–6]. However, unlike somatic X inactivation, the phenomenon in germ cells has not been explained in a way that applies to diverse taxonomic groups with either XY or ZW sex determination. This difficulty has been the weakness of the 'germline-first' hypothesis. We now suggest that a redistribution of spermatogenic genes between the X chromosome and the autosomes, driven by sexual antagonism, could underlie the evolution of germline X inactivation.

X inactivation in the germ cells and in soma

The connection between the two types of inactivation is plausible if the paternal X chromosome is always inactivated in the daughter's soma as shown in Fig. 1. Generally, the germline-first hypothesis is based on three types of observation. First, germline X inactivation is very common taxonomically [3,7] (whereas somatic X inactivation evolved only once in the ancestral mammals). In *Caenorhabditis*, insects and mammals, before and during meiosis the X chromosome is often stained differently from the autosomes and condensed [3,8,9]. Expression data, chromosome translocation results and transgenic experiments [3,10–12] generally confirm this interpretation.

Second, somatic X inactivation appears to be a more elaborate process than germline X inactivation, as it requires a counting mechanism. Richler *et al.* compared the two phenomena [10]; both include the expression of the *Xist* gene in the testis [13], the coating of the XY body by *Xist* RNA [5] and the concentration of the macroH2A macro-histone in the XY compartment [8]. However, unlike the germline X inactivation, somatic X inactivation includes H4 hypoacetylation, and its initiation is dependent on the expression of *Xist*. Even the maintenance of somatic X inactivation appears to follow somewhat different rules to germline X inactivation, with varying degrees of dependence on *Xist* and macroH2A [14].

Third, in birds, where the heterogametic sex is female (chromosomes ZW), there does not appear to be somatic Z inactivation in ZZ males as a means of dosage compensation [2,15]. (The possibility of dosage compensation by other mechanisms has been raised [16], but that is not directly relevant here.) The difference between birds and mammals in X(Z) inactivation can be explained by the 'germline first' hypothesis. In ZW females (birds and

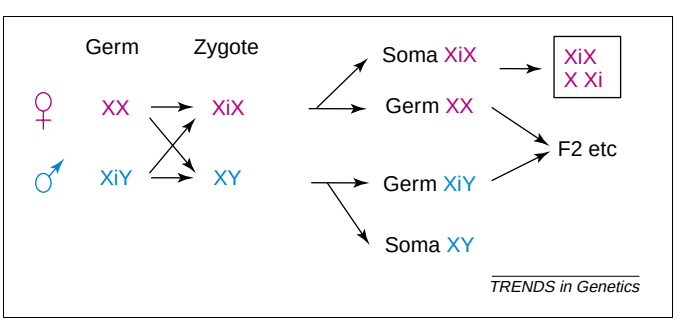

Fig. 1. Possible connections between germline and somatic X inactivation. Xi denotes the inactivated X chromosome, which is passed on from father to daughter. In the extreme case such as the creeping vole [3], the inactivation is further achieved by physical elimination (Xi = O), making the father-to-daughter connection in X inactivation obvious. In female germ cells, Xi is reactivated. In eutherian mammals, an extra step of reactivation and random inactivation in the soma is shown in the box.

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butterflies), there is no indication of Z inactivation during oogenesis [15,17,18] and Z-to-autosome translocations do not result in female sterility. By contrast, in XY males X-to-autosome translocations can cause sterility. In the absence of a mechanism for germline Z inactivation, there might not have been any basis from which the somatic event could have evolved.

Why, then, did the (precocious) Z inactivation not evolve in oogenesis? It might be easier to look first at spermatogenesis during which all chromosomes are condensed. This condensation mechanism could be necessary for packaging the genetic material into the relatively small and highly mobile spermatozoan. With such a mechanism in place, it is easier to adjust the timing and precociously inactivate the X chromosome. An alternative theory is that the egg represents a state of active transcription [19], which is incompatible with condensation and inactivation of chromosomes. Because the general mechanism of chromosome inactivation is absent in birds, they did not evolve to inactivate the Z chromosome selectively and precociously during oogenesis.

Sexual antagonism driving the evolution of germline X inactivation – a hypothesis

If germline X inactivation is a precursor of the somatic X inactivation, what then drove the evolution of the germline event? A necessary condition for precocious X inactivation is the loss of X-linked functions necessary for late (relative to the timing of inactivation) spermatogenic development. This loss presumably has to be accompanied by either a compensating gain on the autosome or the retention of these late functions on the Y chromosome.

What makes it possible for the apparent transfer from the X chromosome of genes involved in late spermatogenesis? The driving force could be sexual antagonism; that is, the genetic changes that are favored in one sex but disfavored in the other [20]. Naturally, autosomal, X- and Y-linked genes would evolve along different paths under sexual antagonism [20]. Autosomal genes are distributed evenly between the two sexes and would not become have specialized sex-linked functions. The Y chromosome, however, spends all of the time in males and should retain only male-specific functions, and X-linked genes spend two-thirds of the time in females. Thus, X is expected to be somewhat 'femininized', whereas Y is expected to be either 'masculinized' or, more likely, degenerated. We refer to the hypothesis of sexual antagonism driving germline X inactivation as the SAXI hypothesis.

We shall classify all mutations into two classes – sexually antagonistic mutations and non-sexually antagonistic ones. The former will be beneficial in one sex and deleterious in the other, whereas the latter will either be advantageous in both sexes, or advantageous in one sex and neutral in the other. Among all developmental processes, gametogenesis is the most likely place for sexual antagonism to be manifested. In general, the more sexually differentiated the process is, the more probable it is that genes controlling it could benefit one sex at the expense of the other. Because late gametogenesis is highly differentiated between sexes, we expect sexual antagonism to be strongest there.

Let us assume that an X-linked gene that functions in late spermatogenesis, as well as some aspects of female development, is duplicated onto an autosome (Fig. 2). A mutation in such a gene is likely to be sexually antagonistic, favoring either female functions to the detriment of late spermatogenesis, or vice versa. The X-linked copy is likely to accumulate mutations favoring female function, even at the expense of males, because it spends two-thirds of the time in females. However, the autosomal copy could become more male-oriented [21]. Although the autosomal copy by itself should be unbiased toward either sex, it could become male-oriented in response to the demasculinization of the X-linked gene by a mechanism similar to the subfunctionalization of duplicated genes [22]. We therefore expect the gradual accumulation of genes involved in late spermatogenesis on the autosomes, concurrent with the demasculinization of their X counterparts. Lastly, gene duplication between the X chromosome and an autosome should equalize the dominance relationship between the two copies. In other words, unlike the more common models on sexual antagonism [20], the SAXI hypothesis is unaffected by the recessivity of X-linked genes.

The predictions of the SAXI hypothesis

The SAXI hypothesis makes three predictions. First, late spermatogenic genes, which are more likely to be sexually antagonistic than others, would move from the X chromosome to the autosomes (Fig. 2). The late stage of spermatogenesis would thus become less and less dependent on the X chromosome, which might even begin to accumulate mutations favoring female functions at the expense of

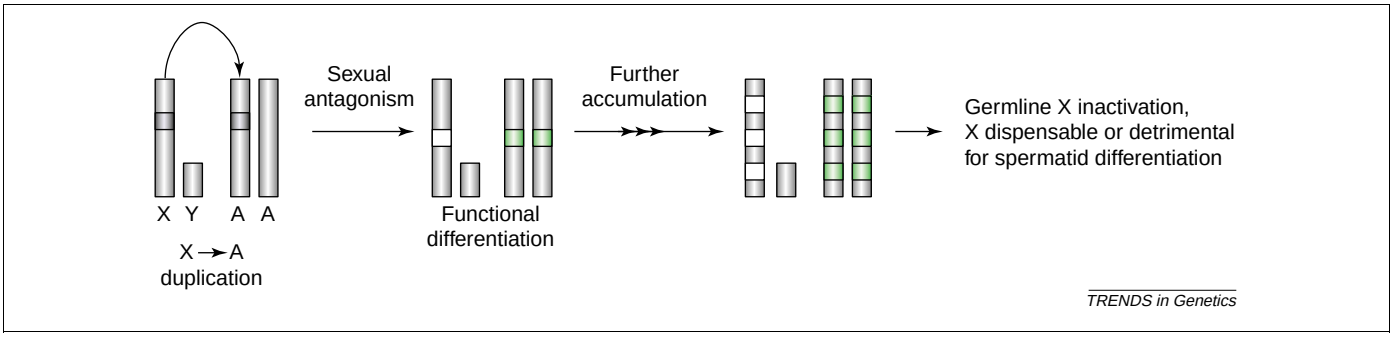

Fig. 2. Gene transposition from the X chromosome to an autosome and the subsequent functional differentiation between the two copies. For convenience, only the male genotype is shown. Black, the ancestral gene; white, X-linked copy of the gene that loses the late-spermatogenic function; green, autosomal copy of the gene that retains the late spermatogenic function.

males. As a consequence, there could be selective pressure to inactivate large segments of the X chromosome early during spermatogenesis.

Second, the gradual evolution of X inactivation might exert pressure to advance the timing of expression of the spermatogenic genes that remain on the X chromosome. Consequently, there should be a greater concentration of the very early spermatogenic genes on the X chromosome than on autosomes.

Third, in the SAXI hypothesis, chromosomal redistribution of gametogenic genes precedes the evolution of X chromosome inactivation. Hence, the redistribution should be observable even in species without germline X inactivation.

Testing the predictions

The expression data

With respect to the first prediction concerning the chromosomal redistribution of spermatogenic genes, the most direct evidence comes from transposed genes and their expression. In mammals, there are four known cases where an X-linked gene has a retrotransposed version on an autosome: Pgk-2 [23], Pdha-2 [24], Zfa [25] and G6pd-2 [26]. In all cases, the X-linked copy has a nearly ubiquitous expression pattern outside of the testis, whereas the autosomal one is expressed during late spermatogenesis. In the case of Pgk-2, a comparison among species suggests that the retrotransposed autosomal copy has evolved from a pattern of wide expression toward spermatogenic specificity [21]. In a systematic survey of retrotransposition in *Drosophila*, Betran *et al.* [27] observed 24 interchromosomal retrotranspositions; in 12 of them the gene involved moved from the X to an autosome, more than twice the expected number. (In a bigger sample of more divergent genes, 63 of the 159 events were from the X to the autosomes, significantly higher than the expected 36.6.) The striking pattern is that 91% of the X-to-autosome retrotranspositions give rise to testicular expression.

Following the convention, Betran *et al.* suggested the escape from germline X inactivation as the cause of this bias, but we propose that X inactivation could be the consequence, rather than the cause, of gene redistribution. We do not suggest that these transpositions were directly involved in germline X inactivation. What these observations show are the evolutionary forces driving the differentiation between the X-linked and autosomal copies after gene transpositions. Because the two processes, X-to-autosome gene transfer and X inactivation, are likely to have evolved gradually, one probably followed the other in a stepwise manner. Moreover, our model does not exclusively require X-to-autosome transposition. Autosome-to-X would be equivalent and, in the proto-XY stage before the massive degeneracy of Y-linked genes, Y-to-autosome transpositions would have provided the same raw materials. In general, the degeneracy of the Y-linked copy and the functional divergence between the X and autosomal copies may leave little trace of homology among chromosomes, but recent observations have managed to show that transposition is an active and ongoing process [28].

Because the direct evidence of gene transposition would gradually lose its strength, a more common observation is the relative abundance of testis-expressing genes on the X versus autosomes. In *Drosophila* and *Caenorhabditis elegans*, there is an apparent paucity of X-linked testis-expressing transcripts [29,30]. Andrews *et al.* [29] found that the density of *Drosophila* genes that are over-expressed in the testis is less than half that on the major autosomes [31], whereas ovary-expressed genes, or genes in general, are more or less evenly distributed among the major chromosomes. In *C. elegans*, the paucity of X-linked spermatogenic genes is even more pronounced – only 3% as many as would have been predicted [30].

The observations on spermatogenic genes of all stages in *Drosophila* and *C. elegans* provide an interesting contrast with the mammalian study of early spermatogenic genes. Wang *et al.* [32] reported that ten out of 25 spermatogonia-specific genes in mouse are X-linked, whereas two at most were expected. (These authors also used the term 'sexual antagonism', but their usage is very different from the meaning in our model. Sexual antagonism in their report concerns the dominance–recessivity relationship, which is not applicable in our model, as addressed earlier.) Recently, Betran *et al.* [27] compiled 21 late spermatogenic genes in mammals. The A:X ratio for these late-acting genes is 21:0, in sharp contrast with the ratio of 15:10 for the early-acting ones [32].

The second prediction of the SAXI hypothesis is therefore corroborated by the mammalian data. For *Drosophila*, there are as yet no expression data on early versus late spermatogenic genes, but the genetic experiments presented below are germane to this issue.

The genetic effect

In a transposon-tagging survey for genes of male sterility, Castrillon *et al.* [33] characterized 58 autosomal mutations for their phenotypic effect, and nearly 80% of them affect late spermatogenic development (defined by the earliest detectable defect during or after meiosis). Only 7–15% of these mutations have an effect on either viability or female fertility. The results are in broader agreement with earlier mutagenic studies on the autosomes using the mutagen EMS. However, of >100 EMS-induced male sterile mutations on the X chromosome of *Drosophila*, none was truly specific to spermatogenesis [34].

We have used *P*-element mutagenesis in a screen for X-linked male sterile mutations in *Drosophila* (E.Y. Xu and C-I. Wu, unpublished). We obtained 20 such mutations and generally attempted to make the procedure comparable with the Castrillon *et al.* report. Mutations were characterized according to their earliest detectable defects in spermatogenesis, as well as their effects on viability and female fertility. We hypothesize that X-linked male sterile mutations would affect early spermatogenic functions more often than the autosomal mutations. Moreover, although some of these early-spermatogenesis mutations would be testis-specific as reported for mouse [32], many others should have pleiotropic effects on viability or female fertility. This proportion is expected to differ between X-linked and autosomal genes (E.Y. Xu, PhD thesis, University of Chicago, 1996). The results are summarized in Table 1 and generally corroborate the predictions.

Table 1. Comparison of male-fertility genes on the X chromosome versus autosomes

	Early spermatogenic defect[a]	Female sterility	Low viability
X-linked genes	60% (12/20)	64% (7/11)[b]	40% (8/20)
Autosomal genes	21% (12/58)	14% (4/28)	7% (2/28)
Fisher's exact test	$P < 0.01$	$P < 0.01$	$P < 0.01$

[a]Early defects of spermatogenesis are all premeiotic.
[b]Only homozygous females with high viability were tested for fertility.

Is the redistribution predicted by the conventional models?

The conventional models for the evolution of germline X inactivation usually invoke protection of the unpaired sex chromosomes and/or suppression of recombination between the sex chromosomes [3,5,7,9]. If the driving force is recombination suppression, we might have expected that the usual mechanisms like chromosome inversion would be sufficient and that species with XO males [35] or achiasmic males (e.g. *Drosophila*) [3,36] would not exhibit germline X inactivation, contrary to the observations.

Moreover in birds and Lepidoptera, ZW females do not inactivate the Z chromosome during oogenesis [17,18]. This is in contrast to predictions from the pairing-recombination models, where a series of steps evolved to compensate for the fitness reduction caused by the previous step. Thus, in these models the X chromosome first evolved to an unpaired (and presumably vulnerable) state and chromosome condensation evolved to protect it. Gene redistribution then evolved to escape this condensation or inactivation.

It is questionable whether evolution could proceed this way. Because X inactivation is a chromosome-level phenomenon involving a large number of genes, the fitness reduction associated with gene silencing should be substantial. However, gene-by-gene redistribution could only offer piece-meal compensation for the loss and, unlike changing the timing of expression, is a slow process pending the rare occurrence of gene transposition. It seems more reasonable that the redistribution of spermatogenic genes should precede, rather than follow, the evolution of germline X inactivation.

Redistribution of gametogenic genes in species without X inactivation

In the SAXI hypothesis, the redistribution of gametogenic genes between X (or Z) and autosomes would occur regardless of the status of germline X inactivation (the third prediction). In the conventional models, the redistribution follows X inactivation. Therefore, the conventional models would not expect a difference in the density of gametogenic genes between Z and autosomes, whereas we predict either an excess of spermatogenic genes or a deficit in oogenic genes on the Z chromosome of birds or butterflies. (Note that, whatever the pattern of distribution of gametogenic genes, the absence of Z-inactivation in ZW systems will still need to be accounted for by the conventional models.) Species in the proto-XY stage that have not evolved germline X inactivation (such as the mosquito [7,37]) or species that have acquired a neo-X chromosome [38] are also relevant. According to the SAXI hypothesis, these species are expected to have a moderate degree of redistribution of spermatogenic genes between the proto-X (or neo-X) chromosome and the autosomes, as long as recombination between the proto-X and proto-Y is absent.

As yet, there are no genome-scale expression data pertaining to gametogenic genes and their chromosomal locations from either the ZW systems or the proto-XY species. It is our hope that the SAXI hypothesis will stimulate some effort in that direction.

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