

# The war of the sex chromosomes

Nathan A. Ellis

Department of Human Genetics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, USA. e-mail: [n-ellis@ski.mskcc.org](mailto:n-ellis@ski.mskcc.org)

With the publication of Ohno's watershed monograph<sup>1</sup>, molecular genetic characterization of the mammalian sex chromosomes has radically altered our view of X-chromosome inactivation and the evolutionary conservation of synteny of the mammalian X chromosome (Ohno's law). X inactivation is the dosage compensation mechanism in mammals whereby during embryogenesis of females a single X-chromosome is genetically silenced. Although most genes are subject to X inactivation, some escape. Moreover, during evolution autosomal genes can be added to the X chromosome and later incorporated to different extents into the inactivation system. A recent report by Karin Jegalian and David Page shows that inactivation of individual X-linked genes can evolve independently in different mammalian lineages<sup>2</sup>. The general correlation between the presence of a functional Y homologue and escape from X inactivation is remarkable; these results confirm the prevailing theory that Y deterioration is the driving evolutionary force behind the dosage compensation mechanism.

The evolution of the dosage compensation mechanism is inextricably interwoven with the evolution of the mammalian sex determination system and the formation

of a functional Y homologue and escape from X inactivation is remarkable; these results confirm the prevailing theory that Y deterioration is the driving evolutionary force behind the dosage compensation mechanism. The evolution of the dosage compensation mechanism is inextricably interwoven with the evolution of the mammalian sex determination system and the formation of the Y chromosome (Fig. 1). The sex chromosomes started out as a pair of ordinary autosomes. Once the Y chromosome was elected to accumulate factors contributing to male fitness, a series of events was initiated which culminated in a puny, anarchic Y chromosome and a tightly controlled, hierarchically regulated X chromosome. Analogous to the Biblical sequence in which order follows chaos, a dosage compensation mechanism developed in response to the mass destruction of Y-encoded homologues. Genetic isolation of the Y led to the accumulation of recessive mutations (a process known as Müller's ratchet) that, in aggregate, reduced male fitness; this in turn 'preserved' the X chromosome to increase its expression. Excessive X-gene expression is presumed to have decreased female fitness until the X-inactivation system evolved to silence the doubly-dosed genes on one of the X chromosomes in females.

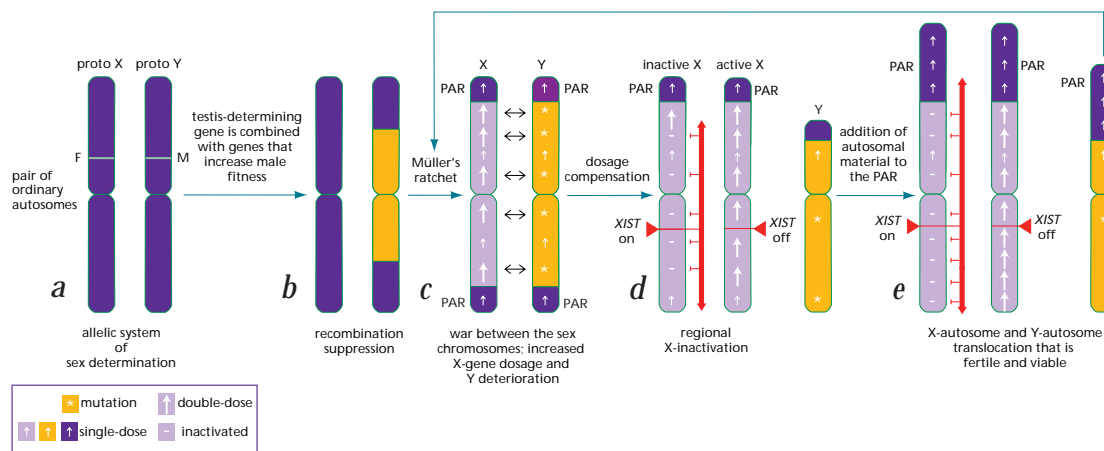
The process by which a single X chromosome is selected for inactivation (Fig. 1) is controlled by a single locus known as *XIST* (ref. 3). Emanating from the *XIST* locus, an inactivation signal is established that spreads along the X chromosome. It was once thought that almost every gene on the X is inactivated; but it is now known that a surprisingly large number (over 20, and possibly many more) escape X inactivation<sup>4,5</sup>. Thirty years ago, only one escapee gene had been localized to the distal tip of the short arm of the X chromosome. Today, we know of many such escapees, proximal as well as distal, and on the long arm as well as on the short—which indicates that spreading of inactivation is a process mediated on a regional or gene-by-gene basis.

During the course of evolution, an ancestor to placental mammals must have escaped a peril resulting from the hemizygous existence of all the X-linked genes. Once this step was accomplished, the female no longer needed two Xs in her somatic cells. Hence, the dosage compensation mechanism by random inactivation of one or the other X evolved.

—Susumu Ohno

mosome is genetically silenced. Although most genes are subject to X inactivation, some escape. Moreover, during evolution autosomal genes can be added to the X chromosome and later incorporated to different extents into the inactivation system. A recent report by Karin Jegalian and David Page shows that inactivation of individual X-linked genes can evolve independently in different mammalian lineages<sup>2</sup>. The general correlation between the pres-

ence of a functional Y homologue and escape from X inactivation is remarkable; these results confirm the prevailing theory that Y deterioration is the driving evolutionary force behind the dosage compensation mechanism. The evolution of the dosage compensation mechanism is inextricably interwoven with the evolution of the mammalian sex determination system and the formation of the Y chromosome (Fig. 1). The sex chromosomes started out as a pair of ordinary autosomes. Once the Y chromosome was elected to accumulate factors contributing to male fitness, a series of events was initiated which culminated in a puny, anarchic Y chromosome and a tightly controlled, hierarchically regulated X chromosome. Analogous to the Biblical sequence in which order follows chaos, a dosage compensation mechanism developed in response to the mass destruction of Y-encoded homologues. Genetic isolation of the Y led to the accumulation of recessive mutations (a process known as Müller's ratchet) that, in aggregate, reduced male fitness; this in turn 'preserved' the X chromosome to increase its expression. Excessive X-gene expression is presumed to have decreased female fitness until the X-inactivation system evolved to silence the doubly-dosed genes on one of the X chromosomes in females.



**The Ys and wherefores of sX chromosome evolution.** Model for the evolution of the mammalian sex chromosomes. **a**, Mammalian sex chromosomes evolved from a pair of ordinary autosomes. At first, sex was genetically determined by a simple diallelic system, F and M, in which the male was the heterogametic sex. **b**, Sex chromosome differentiation began when the proto-Y chromosome accrued at least one additional gene, that together with the M allele, conferred a selective advantage to males. Such combinations of syntenic genes in turn selected for a meiotic system in which recombination between sex chromosomes was suppressed (a region of suppression is represented by the yellow area on the Y). **c**, The inevitable consequence of recombination suppression was the additional accrual by the Y chromosome of recessive mutations (\*) in the many genes unrelated to sexual development by a process known as Müller's ratchet. Recessive mutations in the recombination-suppressed segment are not effectively selected against, and patrilineal inheritance reduces the effective population size of Y genes, resulting in increased genetic drift. The loss in fitness in males that resulted from hemizygosity of more and more X chromosome genes was corrected by increasing the expression of the X genes, thus raising the level of X-gene expression in females. A region has been maintained that is shared by the sex chromosomes (denoted PAR for pseudoautosomal region). Crossing-over is confined to this segment and is required for the segregation of the sex chromosomes in male meiosis. **d**, Inactivation of one X chromosome in females evolved. A single locus, *XIST*, was recruited to initiate and signal genetic silencing of one X chromosome in females (indicated by the larger arrows alongside the X chromosome). PARs in most eutherians are small regions, and in certain circumstances they can be dispensed with altogether (as in marsupials). On the other hand, PARs can be sites to which autosomal material can be translocated (**e**). The newly translocated material then becomes subject to Müller's ratchet and the dosage compensation wars.

Table 1 • X-chromosome inactivation and Y-chromosome deterioration

Gene	Primates		Domesticated mammals		Rodents		Marsupials	
	XCI <sup>a</sup>	Y gene <sup>b</sup>	XCI	Y gene	XCI	Y gene	XCI	Y gene
<i>ZFX</i>	no	yes	no	yes	yes	no <sup>c</sup>	autosomal <sup>d</sup>	
<i>RPS4X</i>	no	yes	yes	no	yes	no	yes	
<i>SMCX</i>	no	yes	yes	no <sup>c</sup>	no	yes	yes	
			no	yes				
			yes	yes <sup>e</sup>				

<sup>a</sup>XCI, X-chromosome inactivation; yes, subject to X inactivation; no, escapes X inactivation; blank, not known. <sup>b</sup>Y gene, functional homologue on the Y chromosome; yes, homologue present; no, homologue absent. <sup>c</sup>In some cases, a Y gene may be present but either nonfunctional (no Y transcripts detected) or expression is reduced in level or restricted (the rodent *Zfy* genes, which are expressed in testis only, have reduced homology to the highly conserved *Zfx* genes, suggesting perhaps an evolving gene function). <sup>d</sup>*ZFX* is autosomal in marsupials and in monotremes, as described in the text. <sup>e</sup>Gene is present, but expression unknown.

### Paring PARS

On the human sex chromosomes, there is a segment of 2.6 million base pairs on the distal tips of the short arms (PAR1) where, contrary to the usual prohibition, crossing-over during male meiosis is not only allowed but required for successful segregation of the X and the Y chromosomes. PAR1 genes are present in two doses in both males and females, and they escape X-inactivation. As such, it seems justifiable that PAR genes are not subject to Ohno's legendary law. Similarly, a second PAR (PAR2) exists on the distal tips of the long arms where at least one gene is known to disobey Ohno's law. Even more illuminating is the fact that a huge portion of the eutherian X chromosome originated as chromosome material that was translocated in several pieces from several different autosomes sometime since the evolutionary paths of eutherians and metatherians separated about 150 million years ago. The current human PAR1 is a remnant of what was once a much larger PAR (ref. 6). How did it shrink? As ever, the expression and integrity of the sex-chromosome genes provide a clue.

One formerly pseudoautosomal gene, human *STS*, is only partially X-inactivated, whereas its Y homologue has become a non-functional pseudogene. Similarly, human *PRKX* escapes X inactivation; its Y homologue is intact but is expressed at a lower level than the X homologue. Thus, the dual processes of Y deterioration and X inactivation have to different extents integrated the formerly pseudoautosomal genes into the sex chromosome system. What remains are X genes in various intermediate evolutionary states. At one end of the spectrum are X genes that are subject to inactivation and have lost their functional Y chromosome counterparts, and at the other end are X genes that escape X inactivation and maintain functional Y counterparts.

Jegalian and Page have studied the genes that, at least in humans, have resisted the dual processes of Y deterioration and X

inactivation. As a suitable surrogate for inactivation status, they analysed the methylation levels in the promoter regions of three genes, *ZFX*, *RPS4X* and *SMCX*, in 19 eutherian species representing 11 orders (Table 1). The results were striking: *ZFX* escapes X-inactivation, and a functional homologue, *ZFY*, is maintained on the Y chromosome in all mammals except rodents. In rodents, *ZFX* is inactivated and the expression level of *ZFY* is reduced and restricted to germ cells. *RPS4X* escapes X inactivation only in the primate lineage; everywhere else, *RPS4X* is X-inactivated. Concomitantly, a functional *RPS4Y* homologue is maintained in the primate lineage only. Finally, *SMCX* is X-inactivated in some species and escapes inactivation in others. *SMCY* gene sequences are present in many species, but the available evidence suggests that in species in which the X gene is inactivated, Y gene function is reduced. In mouse, inactivation of *SMCX* occurs in embryogenesis but the gene partially reactivates during later stages of development<sup>7</sup>. Mouse *SMCX* appears to be in an intermediate state in which Y function is normal but X inactivation has not been completely successful.

These data demonstrate that the X-inactivation system has embraced these three genes in some mammalian species but not in others, and, wherever X inactivation is observed, the expression of the homologue on the Y chromosome is either reduced, or the Y gene is lost entirely. If X inactivation were driving these evolutionary events, functional Y genes with X-inactivated homologues would be more frequent. Functional human Y genes have been thoroughly studied, and it is remarkable how consistently their expression is either testis-specific or, alternatively, ubiquitous but with a functional X homologue that escapes X-inactivation<sup>8</sup>. Many are the defunct Y genes that have X homologues that escape X inactivation, and many are the X-inactivation escapees with no Y homologue at all. From all the available evidence, we can conclude that, for the most part, Y deteriora-

tion is the driving evolutionary force. The mouse *SMCX* gene, however, tells a cautionary tale; it shows that X inactivation can be the driving evolutionary force.

X-inactivation escapees *RPS4X* and *SMCX* were present on the X chromosome ancestral to eutherians and metatherians and *ZFX* was one of those genes translocated in the eutherian lineage to the X chromosome from an autosome<sup>6</sup>. Most of their former colleagues were driven off the Y chromosome long ago. What makes these genes more resistant to the dual processes of Y deterioration and X inactivation? Perhaps they resist the turns of Müller's ratchet because they are dosage-sensitive genes. In other words, mutation or X inactivation of one copy of these genes may result in negative phenotypic effects (for example, growth or fertility defects). This hypothesis could be testable by generating a mouse deficient of *SMCX*. Supporting it is the fact that somatic features resembling those of people with Turner syndrome have been observed in females with XY gonadal dysgenesis who have deletions of *ZFX* and *RPS4X*, suggesting that these genes may indeed be dosage sensitive. How Y deterioration and X inactivation would capture dosage-sensitive genes in some mammalian lineages but not others is unknown; perhaps the two processes occasionally pounce simultaneously on a gene, or perhaps modulations of autosomal genes that control dosage sensitivity suddenly confer dosage resistance.

The mammalian X inactivation system has turned out to be far more malleable and adaptive than originally imagined. Inactivation is controlled on both a chromosome-wide and a gene-regional basis, and X-inactivation status continues to evolve even today as new genes are shuffled into the PAR and later captured by the non-recombining part of the Y chromosome. Once capture has been effected, Müller's ratchet proves to be an exceptionally strong force—to which the vast number of Ohno-obedient X-inactivated genes testify. While the Y chromosome stands accused of violence to many decent, hard-working genes, at least some of us can be thankful that, although it may not be much, it's good at what it does. □

- Ohno, S. (Springer-Verlag, Berlin, 1967).
- Jegalian, K. & Page, D.C. *Nature* **394**, 776–780 (1998).
- Carrel, L. & Willard, H.F. *Nature Genet.* **19**, 211–212 (1998).
- Disteche, C. *Trends Genet.* **11**, 17–22 (1995).
- Brown, C., Carrel, L. & Willard, H.F. *Am. J. Hum. Genet.* **60**, 1333–1343 (1997).
- Graves, J.A.M., Disteche, C.M. & Toder, R. *Cytogenet. Cell Genet.* **80**, 94–103 (1998).
- Lingenfelter, P.A. et al. *Nature Genet.* **18**, 212–213 (1998).
- Lahn, B.L. & Page, D.C. *Science* **278**, 675–680 (1997).