[Cell Cycle 3:7, 873-875; July 2004]; ©2004 Landes Bioscience

Perspectives

Sex Chromosomes and Male Functions

Where Do New Genes Go?

Esther Betrán[1]
J.J. Emerson[2]
Henrik Kaessmann[3]
Manyuan Long[2,*]

[1]Department of Biology; University of Texas at Arlington; Arlington, Texas USA

[2]Department of Ecology and Evolution; University of Chicago; Chicago, Illinois USA

[3]Center for Integrative Genomics; BEP; University of Lausanne; Lausanne, Switzerland

*Correspondence to: Manyuan Long; Department of Ecology and Evolution; University of Chicago; Chicago, Illinois 60637 USA; Tel.: 773.702.0557; Fax: 773.702.9740; Email: mlong@midway.uchicago.edu

Received 05/01/04; Accepted 05/05/04

Previously published online as a *Cell Cycle* E-Publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=960

KEY WORDS

X chromosome, retroposition, duplication, sexual dimorphism, sex-biased gene, mammals

ABSTRACT

The position of a gene in the genome may have important consequences for its function. Therefore, when a new duplicate gene arises, its location may be critical in determining its fate. Our recent work in humans, mouse, and *Drosophila* provided a test by studying the patterns of duplication in sex chromosome evolution. We revealed a bias in the generation and recruitment of new gene copies involving the X chromosome that has been shaped largely by selection for male germline functions. The gene movement patterns we observed reflect an ongoing process as some of the new genes are very young while others were present before the divergence of humans and mouse. This suggests a continuing redistribution of male-related genes to achieve a more efficient allocation of male functions. This notion should be further tested in organisms employing other sex determination systems or in organisms differing in germline sex chromosome inactivation. It is likely that the selective forces that were detected in these studies are also acting on other types of duplicate genes. As a result, future work elucidating sex chromosome differentiation by other mutational mechanisms will shed light on this important process.

X and Y mammalian chromosomes likely originated through morphological and functional differentiation from a pair of ancestral autosomal chromosomes.[1] Two main processes are implicated in sex chromosome morphological differentiation: most of the Y chromosome has degenerated as a consequence of suppression of crossing-over, and the X chromosome has developed dosage compensation.[2] The current models of how this occurs depict a system that began with the evolution of a male-determining gene on the Y chromosome. The subsequent degeneration occurs through lack of recombination by Muller's ratchet, that is, the stochastic loss of the chromosomes that carry the smallest number of deleterious mutations in a finite population,[3] background selection resulting from decreased population size due to elimination of chromosomes that carry strongly deleterious mutations,[2] and the fixation of deleterious alleles by hitchhiking with selectively favourable mutations.[2-5] While the Y chromosome is degenerating, dosage compensation takes place in mammals via X inactivation in female somatic cells, which ensures equal levels of expression of genes on the X in males and females.[2] In fruit flies dosage compensation is achieved by increased transcription of the male X chromosome.[6] In both systems, the X chromosome is precociously inactivated in the male germline.[7-9] The reasons for this are not well understood, but it has been proposed that the condensation of the X chromosome in the male germline, resulting in the inactivation of X-linked genes, might serve to protect the chromosomes from damage or apoptosis during spermatogenesis.[9,10]

We analyzed duplicate genes generated by retroposition in order to examine patterns of gene movement by duplication in morphologically differentiated genomes.[11] Retroposition is the mechanism whereby a processed mRNA is reverse transcribed and reintegrated into the genome. The LINE (L1 retrotransposon in humans) enzymatic machinery is known to play a role in this process.[12] LINEs are abundant endogenous mobile elements that transpose via reverse transcription of their own transcript.[13] This process likely occurs through target-primer reverse transcription and repairing wherein a LINE encoded endonuclease nicks the DNA, leaving a 3'OH that primes the reverse transcription of the LINE RNA,[13,14] after which the synthesis of the other strand and the repair takes place.[15] Esnault et al.[12] demonstrated that new processed copies of genes can be generated using LINE enzymatic machinery in a similar way. Moran et al.[16] have also shown that LINEs can help to retropose sequences derived from their 3' flanking region to new genomic locations. The direction of copying can be inferred from sequence comparison between the parental gene and the retrogene: the processed copy is intronless (young copies may also display a 3' poly-A tract and flanking direct repeats derived from the molecular

process of retroposition), whereas the parental gene from which it is derived usually contains introns.

This approach revealed intriguing patterns of gene movement in the human, mouse, and *Drosophila* genomes: the mammalian X chromosome has both generated and recruited an unexpectedly high number of functional duplicates,[11] whereas *Drosophila* exhibits unidirectional excess of retroposition from X to autosomes.[17] To distinguish between mutational biases and selective forces that may have shaped movement patterns in the human genome, we separately analyzed functional retroposed genes (retrogenes) and retroposed pseudogenes (retropseudogenes[11]). Retropseudogenes are retrocopies that are clearly nonfunctional due to disablements such as premature stop codons. We demonstrated that the export of functional retrogenes from the X chromosome is incompatible with mutational bias,[11] because retropseudogenes did not show excess movement out of the X. Interestingly, most of the autosomal copies originating from X-linked genes exhibited testis-biased expression. Thus, we inferred that the export of genes by the X is likely driven by natural selection to attain male germline function. In *Drosophila*, the LINE-like retrotransposons are randomly distributed on the X chromosome and autosomes[18] suggesting a random mutational process in this species as well.

Cases of individual genes being copied from the X chromosome to different genomic locations more suited to a particular function have been described (i.e.,[19-22] and see ref. 11, supplementary materials). These cases involved genes with male germline functions. It has been suggested that the presence of X inactivation in male germline cells could explain the preference of these copies for the autosomes.[19-22] According to this model, the precocious inactivation of the X chromosome might constitute a selective pressure for the export of functional retroposed gene copies, if such functions are desirable during male meiosis and fertility is improved by having the genes encoding these functions in autosomes that are not involved in the inactivation and therefore can express the functions. It has been postulated that in the case of *Pgk2*, the existence of the new copy could have made X inactivation possible.[20] In addition, there might also be some advantage of being in an autosome during haploid stages.[23] A model of sexual antagonism called SAXI model, proposes that newly duplicated genes with male germline function would be likely found on the autosomes because a way for the males of escaping antagonistic effects of a gene on the X chromosome is to make a copy in an autosome and inactivate the original copy. According to this model, the X chromosome spends 2/3 of its evolutionary time in female and 1/3 of the time in male, therefore, X would become "demasculinized" and drive male-related genes into autosomes.[24] The third model has been developed based on the dominance of the sex-related genes.[25] A theoretical analysis of population genetics on the rate of evolution of autosomes and X chromosomes[26] showed that if the sex-related mutation is dominant or partially dominant, the probability of its fixation in natural population is higher when it is on autosomes than X-linkage. Consistent with our observations, therefore, more male-related genes should be distributed on autosomes.

Although the aforementioned models are consistent with our observation, no single model can account for all aspects of observed patterns related to gene movement. For example, the X inactivation hypothesis can explain the gene movement in male germline cells but offers little to explain the excess of the genes that are specifically expressed in accessory glands of *Drosophila*[27] and are not associated with meiosis. However, whatever the mechanism, one consequence of such directional gene movement from the X chromosome is obvious: genes with male germline function would be enriched in autosomes after sufficient evolutionary time. This prediction is well corroborated by *Drosophila* expression analysis using DNA microarray technology[26,28] and for many individual genes.[17,25-28] Male germline genes also show a strong preference for autosomes in *C. elegans*.[29] However, it was observed that during mouse spermatogenesis, there is an abundance of X-linked genes expressed in spermatogonia (mitotic cells).[30] We believe that our findings are not contradictory to this observation, because the two investigations address different stages of spermatogenesis.[17,31] It appears that the identified mouse genes in reference 30, observed from the early stage (mitotic cells) of spermatogenesis, are expressed prior to X inactivation.

A difference between mammals and *Drosophila* is that the human X chromosome also shows an excess recruitment of retroposed genes, contrary to the *Drosophila* X chromosome. The number of retropseudogenes entering the X chromosome is nearly twice that predicted by a random model, illustrating that a mutational bias exists. However, this only explains part of the observed pattern;[11] the excess beyond the predicted number that is contributed by the mutational bias may be explained by selection acting on recessive advantageous retropositions to the X that are beneficial when hemizygous in males. This is consistent with the observed paucity of female expression among the retrogenes entering the X.

Is retroposition the only way by which male genes are relocated in the genome? Although only retrogenes were studied in humans, mouse and *Drosophila*, there is no reason to assume that the evolutionary force of natural selection should be acting only on retroposed genes. Interestingly, the Y chromosome, which is recruiting genes with male-specific function, has not restricted its recruitments to retrogenes; other types of duplications (i.e., transpositions) have also taken place.[33] Therefore, it will be interesting to elucidate to what extent other types of gene duplications/rearrangements (e.g., segmental duplications and chromosomal translocation) have contributed to the relocation of genes with male function.

Dating of the retrogenes allowed us to infer that many duplication events occurred in the ancestor of eutherian mammals,[11] indicating an ancient origin of this gene movement process. However, the process also appears to be an ongoing characteristic of eutherian X evolution, as some retroposed genes that originated after the human mouse split on the human lineage show high sequence similarity to the parental copies.[11] This suggests that the mammalian genome is still in the process of repartitioning the genome so that male functions can be efficiently expressed.

With the addition of both experimental and computational genomics to the toolbox for elucidating the differentiation of sex chromosomes, there are indeed many exciting avenues worth of further pursuit. With respect to gene traffic between sex chromosomes and autosomes, there are a number of prospects, both genomically and genetically. In order to understand the selective pressures that might constrain evolution of male genes on the X chromosome, one important avenue will be to investigate other mechanisms of avoiding inactivation. Experiments to approach this question would be valuable: (i) carefully determining which regions of the X chromosome are precociously inactivated and which are not, in order to evaluate whether sex specific function is conditioned by not only X chromosome linakge but also by their positions on the X chromosome, (ii) comparing the expression profiles of X-linked genes to autosomal genes during stages of spermatogenesis before X inactivation in order to determine if overexpression prior to inactivation can compensate for subsequent silencing, as suggested by the over-representation of

X-linked genes in spermatogenic mitosis.[30] Finally, an important goal of future research should be to distinguish between alternate hypotheses that explain the selective pressures. One convenient system in which to carry out such similar genomic studies would be those heterogametic species that have different patterns of sex chromosome inactivation or potential for sexual antagonism.

References

1. Ohno S. Sex chromosomes and sex-linked genes. Berlin: Springer, 1967.
2. Charlesworth B. The evolution of chromosomal sex determination and dosage compensation. Curr Biol 1996; 6:149-62.
3. Charlesworth B. Model for evolution of Y chromosomes and dosage compensation. Proc Natl Acad Sci USA 1978; 75:5618-22.
4. Rice WR. Genetic hitchhiking and the evolution of reduced genetic activity of the Y sex chromosome. Genetics 1987; 116:161-7.
5. Bachtrog D. Evidence that positive selection drives Y-chromosome degeneration in *Drosophila* miranda. Nature Genet 2004; 36:518-22.
6. Baker BS, Gorman M, Marin I. Dosage compensation in *Drosophila*. Annu Rev Genet 1994; 28:491-521.
7. Ayoub N, Richler C, Wahrman J. Xist RNA is associated with the transcriptionally inactive XY body in mammalian male meiosis. Chromosoma 1997; 106:1-10.
8. Lifschytz E, Lindsley DL. The role of X-chromosome inactivation during spermatogenesis (*Drosophila*-allocycly-chromosome evolution-male sterility-dosage compensation). Proc Natl Acad Sci USA 1972; 69:182-6.
9. Richler C, Soreq H, Wahrman J. X inactivation in mammalian testis is correlated with inactive X-specific transcription. Nature Genet 1992; 2:192-5.
10. McCarrey JR, Watson C, Atencio J, Ostermeier GC, Marahrens Y, Jaenisch R, et al. X-chromosome inactivation during spermatogenesis is regulated by an Xist/Tsix-independent mechanism in the mouse. Genesis 2002; 34:257-66.
11. Emerson JJ, Kaessmann H, Betrán E, Long M. Extensive gene traffic on the mammalian X chromosome. Science 2004; 303:537-40.
12. Esnault C, Maestre J, Heidmann T. Human line retrotransposons generate processed pseudogenes. Nat Genet 2000; 24:363-7.
13. Kazazian Jr HH, Goodier JL. LINE drive. Retrotransposition and genome instability. Cell 2002; 110:277-80.
14. Luan DD, Korman MH, Jakubczak JL, Eickbush TH. Reverse transcription of R2Bm RNA is primed by a nick at the chromosomal target site: A mechanism for nonLTR retrotransposition. Cell 1993; 72:595-605.
15. Gilbert N, Lutz-Prigge S, Moran JV. Genomic deletions created upon LINE-1 retrotransposition. Cell 2002; 110:315-25.
16. Moran JV, DeBerardinis RJ, Kazazian Jr HH. Exon shuffling by L1 retrotransposition. Science 1999; 283:1530-4.
17. Betrán E, Thornton K, Long M. Retroposed new genes out of the X in *Drosophila*. Genome Res 2002; 12:1854-9.
18. Kaminker JS, Bergman CM, Kronmiller B, Carlson J, Svirskas R, Patel S, et al. The transposable elements of the *Drosophila melanogaster* euchromatin: A genomics perspective. Genome Biol 2002; 3:84-4.
19. McCarrey JR. Evolution of tissue-specific gene expression in mammals: How a new phosphoglycerate kinase was formed and refined. BioScience 1994; 44:20-7.
20. Hendriksen PJ, Hoogerbrugge JW, Baarends WM, de Boer P, Vreeburg JT, Vos EA, et al. Testis-specific expression of a functional retroposon encoding glucose-6-phosphate dehydrogenase in the mouse. Genomics 1997; 41:350-9.
21. Kleene KC, Mulligan E, Steiger D, Donohue K, Mastrangelo MA. The mouse gene encoding the testis-specific isoform of Poly(A) binding protein (Pabp2) is an expressed retroposon: Intimations that gene expression in spermatogenic cells facilitates the creation of new genes. J Mol Evol 1998; 47:275-81.
22. Wang PJ, Page DC. Functional substitution for TAF(II)250 by a retroposed homolog that is expressed in human spermatogenesis. Hum Mol Genet 2002; 11:2341-6.
23. Hendriksen PJM. Do X and Y spermatozoa differ in proteins? Theriogenology 1999; 52:1295-307.
24. Wu C-I, Xu EY. Sexual antagonism and X inactivation - the SAXI hypothesis. TIGS 2003; 19:243-7.
25. Ranz JM, Castillo-Davis CI, Meiklejohn CD, Hartl DL. Sex-dependent gene expression and evolution of the *Drosophila* transcriptome. Science 2003; 300:1742-5.
26. Charlesworth B, Coyne JA, Barton NH. The relative rate of evolution of sex chromosomes and autosomes. Am Nat 1987; 130:113-46.
27. Swanson WJ, Clark AG, Waldrip-Dail HM, Wolfner MF, Aquadro CF. Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. Proc Natl Acad Sci USA 2001; 98:7375-9.
28. Parisi M, Nuttall R, Naiman D, Bouffard G, Malley J, Andrews J, et al. Paucity of genes on the *Drosophila* X chromosome showing male-biased expression. Science 2003; 299:697-700.
29. Reinke V, Smith HE, Nance J, Wang J, Van Doren C, Begley R, et al. A global profile of germline gene expression in *C. Elegans*. Mol Cell 2000; 6:605-16.
30. Wang PJ, McCarrey JR, Yang F, Page DC. An abundance of X-linked genes expressed in spermatogonia. Nature Genet 2001; 27:422-6.
31. Fujii T, Tamura K, Masai K, Tanaka H, Nishimune Y, Nojima H. Use of stepwise subtraction to comprehensively isolate mouse genes whose transcription is up-regulated during spermiogenesis. EMBO Rep 2002; 3:367-72.
32. Lercher MJ, Urrutia AO, Hurst LD. Evidence that the human X chromosome is enriched for male-specific but not female-specific genes. Mol Biol Evol 2003; 20:1113-6.
33. Saxena R, Brown LG, Hawkins T, Alagappan RK, Skaletsky H, Reeve MP, et al. The *DAZ* gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. Nat Genet 1996; 14:292-9.