Current Biology
Dispatches

Meiotic Sex Chromosome Inactivation: Compensation by Gene Traffic

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http://dx.doi.org/10.1016/j.cub.2017.05.077

It has been widely observed that sex-related genes are copied from sex chromosomes and inserted into autosomes, a process termed 'gene traffic'. However, the adaptive significance of this phenomenon is unclear. Now, direct evidence has been provided that gene traffic may allow functional compensation during meiotic sex chromosome activation.

Sex chromosomes, soon after their origination from autosomes, evolve such that the sex-related genes they harbor tend to be copied and moved to autosomes eventually over time [1,2]. Gene traffic — the biased copying of sex-related genes from sex chromosomes onto autosomes — is widely observed in mammals [1,2] as well as other metazoans such as *Drosophila* [3,4], mosquitoes [5], stalk-eyed flies [6], and silk worms [7]. This extensive export of sex-related genes to autosomes profoundly transformed the chromosomal localization of genes and seems to be driven by natural selection [1,3,8]. However, the underlying mechanisms fueling adaptation are less apparent, though the pronounced excess of duplicates with testis-biased expression offers clues.

One theory explaining gene traffic relates to the process of meiotic sex chromosome inactivation (MSCI), which refers to the silencing of gene expression from the sex chromosomes that accompanies the condensation of the X–Y body during male meiosis [9,10]. MSCI is thought to provide a protective function by shielding the sex chromosomes from damaging recombination in their nonhomologous regions [11]; however, it can also be interpreted more naturally as a by-product of the protective process that suppresses recombination, based on the known non-coding RNA-based molecular mechanism involved in the process [10].

X-inactivation, however it arises, has the potential to create a suboptimal environment for X-linked genes that are important for sperm production. Consequently, escape from X-inactivation (EXI) to an environment less hostile to male meiosis was the dominant hypothesis for the evolutionary mechanism driving gene traffic [1,3,12–14], though its importance was not universally supported [15]. However, MSCI might also arise to resolve genetic conflicts stemming from mutations that benefit one sex and harm the other (i.e., sexual antagonism) [16]. Sexual antagonism could be particularly prevalent in the germline given the highly sexually dimorphic nature of mammalian gametes. Mutations favoring females can lead to feminization of the X-chromosome, which spends most of its time in females. Compensation of mutations that disfavor males could then be accomplished by duplications to autosomes [16]. As genes carrying out male functions gradually accumulate on autosomes, their X-linked counterparts are freed from the need to provide male functions, and can undergo X-inactivation. In this scenario, sexual antagonism drives X-inactivation (SAXI). Thus, the SAXI hypothesis predicts that many genes copied from the X-chromosome to autosomes have diverged in function, making it unlikely that they complement each other. In contrast, the EXI hypothesis makes no such prediction. In support of the EXI hypothesis, a study reported in a recent issue of *Current Biology* by Jiang *et al.* [17] demonstrates that the X-linked gene *RPL10*, encoding a ribosomal protein, and its autosomal copy *RPL10L* can functionally compensate for each other.

While *RPL10* was present in the ancestor of all eukaryotes more than one billion years ago, its paralogous copy, *RPL10L*, originated less than 160 million years ago in the common ancestor of eutherian mammals (Figure 1A). The *RPL10L* gene originated via retroposition of a transcript of the multi-exon X-linked *RPL10* gene, which was reverse-transcribed and reintegrated into the genome, resulting in the loss of all introns in the copy [18,19]. RPL10, like many other ribosome proteins, is among the most conserved mammalian proteins [20], exhibiting only a single conservative substitution in the human lineage out of its 214 residues [17]. The retrogene *RPL10L* is also highly conserved, with its encoded protein showing 98% similarity to orthologues in dogs and mice [17]. This is surprising because duplicate genes, especially retrogenes, frequently evolve quickly compared to their ancestors due to selection for more divergent functions in the new genomic environment. Such sequence conservation in both copies offers an exciting opportunity to test the EXI hypothesis.

Jiang *et al.* employ an impressive battery of functional approaches to address three questions essential to understanding duplicate gene pairs, ultimately leading to a strong test of the EXI hypothesis. These questions include, firstly, what are the molecular and cellular functions of RPL10L and what role does it play in organismal fitness? Secondly, how does expression of *RPL10*, the X-linked parental copy, respond to MSCI operating on the X chromosome, and how does this affect its molecular and cellular functions? And, thirdly, can *RPL10L* functionally compensate for *RPL10* if the latter is silenced during MSCI?

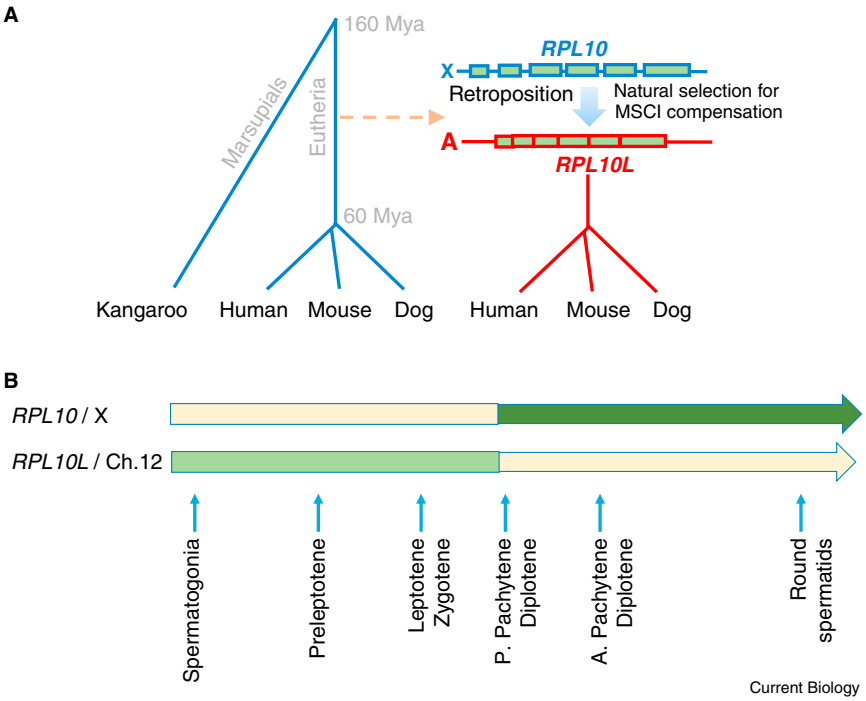

Figure 1. Escape from MSCI by gene duplication.
(A) Depicted is the process of RNA-based duplication of *RPL10* (blue lineages) leading to origination of *RPL10L* in a eutherian ancestor between 60 and 160 million years ago (mya). The retroposition process generated an intronless retrogene, *RPL10L*, in a new position in chromosome 12, which is not affected by MSCI. This new retrogene today has been vertically passed into various eutherian species, exemplified by human, dog and mouse (red lineages) in the simplified gene tree that only shows three eutherian and one metatherian species (Kangaroo). (B) Jiang *et al.* [17] found that the duplicates are expressed in a complementary pattern in which the X-linked *RPL10* was found to be expressed in spermatogonia and early stages of meiosis (preleptotene, leptotene and zygotene) before being silenced afterwards by MSCI on the X (dark green), while the X-derived, autosomal *RPL10L* starts to express in pubertal pachytene and continues to be expressed in subsequent meiosis stages and round spermatids (light yellow). The consequences of the compensated expression on the protein function, cellular reproductive functions and male fertility are examined using CRISPR/Cas9 knockout of *RPL10L* and rescue by *RPL10*.

To understand the role of *RPL10L* in spermatogenesis, CRISPR/Cas9 technology was used to generate *RPL10L*-deficient mouse strains. Strains homozygous for the deficient allele ($RPL10L^{-/-}$) produced viable mice, but males were infertile in mating tests, showed significantly smaller testes, and possessed epididymides devoid of sperm. However, heterozygotes ($RPL10L^{+/-}$) or mice lacking a deficiency ($RPL10L^{+/+}$) showed no such deficits. This suggests that *RPL10L*, the retroposed duplicate of *RPL10*, evolved functions essential for spermatogenesis. While prophase I progresses normally in deficient males, they exhibit substantially fewer spermatocytes in metaphase I, indicating an arrest of spermatogenesis in the transition from prophase to metaphase in meiosis I (Figure 1B). *RPL10L* is the first X-derived retrogene known to be required for meiotic progression during spermatogenesis, enabling a direct test of its hypothetical compensation effects in MSCI.

Proteomic quantification revealed that several proteins known to be required for progression from prophase I to metaphase I are downregulated in mutant homozygotes ($RPL10L^{-/-}$), including NEK2, HSPA2, CCNA1, PLK1 and CKS2. Morever, 97% of ribosomal proteins quantified in the dataset were down-regulated. Unsurprisingly, this massive downregulation of ribosomal proteins coincides with lower ribosome yields in western blots. These results suggest that absence of RPL10L is sufficient to disrupt biosynthesis of ribosomes. Expression analysis of *RPL10L*'s X-linked parent, *RPL10*, confirms that, while it is broadly expressed at similar levels in most tissues in both males and females, its transcription abruptly diminishes in late spermatocytes (pachytene and later), indicating that it is subject to transcriptional silencing by MSCI. RNAi knockdown of RPL10 in human cell lines leads to deficiencies of ribosomes and polysomes and ultimately G1 cell-cycle arrest, showing that RPL10 is an important housekeeping gene necessary for ribosome biogenesis and cell proliferation.

Thus, even though RPL10 and RPL10L are expressed in complementary tissues, mutations that knock out RPL10L and knock down RPL10 lead to mechanistically similar phenotypes resulting from disruption of ribosome biogenesis. This apparent overlap of function suggested to the authors that the two gene copies might actually compensate for each other's loss. Indeed, when endogenous RPL10 is disrupted in human cell lines, ectopically expressed RPL10L compensates for the loss. In the other direction, expression of transgenic RPL10–mCherry in mice under control of the *RPL10L* promoter in $RPL10L^{-/-}$ males mostly compensates for the retrogene's loss, permitting $RPL10L^{-/-}$;RPL10–mCherry males to sire more than 85% the number of pups that $RPL10L^{+/-}$ heterozygotes do.

Taken together, the broad expression pattern of RPL10 contrasted with the testis-specific expression of RPL10L combined with their high similarity and ability to functionally compensate for each other strongly supports the EXI over the SAXI hypothesis. While the incomplete restoration of fertility of $RPL10L^{-/-}$; RPL10–mCherry males holds out the possibility that RPL10 and RPL10L have diverged in function, thereby supporting the SAXI hypothesis, one final observation makes even this possibility less likely. When the seminiferous tubules of $RPL10L^{-/-}$;RPL10–mCherry males were examined, ~27% exhibited defects. In an interesting coincidence, ~27% of seminiferous tubules also failed to express RPL10–mCherry protein. Thus, the incomplete compensation may merely stem from mosaic expression. However, the four times higher expression of X-linked *RPL10* in ovary than in testis suggested a diverged female function, in accordance with a prediction of the SAXI hypothesis. Future work on this retrogene pair addressing this minor uncertainty would permit a final definitive test of

whether they are functionally interchangeable.

This work is noteworthy because it synthesizes competing predictions made from evolutionary genomics with careful and thorough functional genetics studies, and shines a light on the functional causes driving natural selection in determining the fate of a new gene. The functionally compensational nature of this retrogene is likely not peculiar to retroposition [8], as shown in an analysis of *Drosophila* duplicate genes [4]. Indeed, we expect other mechanisms leading to new gene duplicates, like non-allelic homologous recombination, to be subject to the very same forces that the authors elucidate here.

REFERENCES

1. Emerson, J.J., Kaessmann, H., Betrán, E., and Long, M. (2004). Extensive gene traffic on the mammalian X chromosome. Science *303*, 537–540.
2. Zhang, Y.E., Vibranovski, M.D., Landback, P., Marais, G.A., and Long, M. (2010). Chromosomal redistribution of male-biased genes in mammalian evolution with two bursts of gene gain on the X chromosome. PLoS Biol. *8*, e1000494.
3. Betrán, E., Thornton, K., and Long, M. (2002). Retroposed new genes out of the *X* in *Drosophila*. Genome Res. *12*, 1854–1859.
4. Vibranovski, M.D., Zhang, Y., and Long, M. (2009). General gene movement off the X chromosome in the Drosophila genus. Genome Res. *19*, 897–903.
5. Toups, M.A., and Hahn, M.W. (2010). Retrogenes reveal the direction of sex-chromosome evolution in mosquitoes. Genetics *186*, 763–766.
6. Baker, R.H., and Wilkinson, G.S. (2010). Comparative genomic hybridization (CGH) reveals a neo-X chromosome and biased gene movement in stalk-eyed flies (Genus *Teleopsis*). PLoS Genet. *6*, e1001121.
7. Wang, J., Long, M., and Vibranovski, M.D. (2012). Retrogenes moved out of the Z chromosome in the silkworm. J. Mol. Evol. *74*, 113–126.
8. Long, M., Vibranovski, M., and Zhang, Y. (2012). Evolutionary interactions between sex chromosomes and autosomes. In Rapidly Evolving Genes and Genetic Systems, R.S. Singh, J. Xu, and R.J. Kulathinal, eds. (Oxford University Press).
9. Richler, C., Soreq, H., and Wahrman, J. (1992). X inactivation in mammalian testis is correlated with inactive X–specific transcription. Nature Genet. *2*, 192–195.
10. Turner, J.M. (2015). Meiotic silencing in mammals. Annu. Rev. Genet. *49*, 395–412.
11. McKee, B.D., and Handel, M.A. (1993). Sex chromosomes, recombination, chromatin conformation. Chromosoma *102*, 71–80.
12. Shiao, M.S., Khil, P., Camerini-Otero, R.D., Shiroishi, T., Moriwaki, K., Yu, H.T., and Long, M. (2007). Origins of new male germ-line functions from X-derived autosomal retrogenes in the mouse. Mol. Biol. Evol. *24*, 2242–2253.
13. Bradley, J., Baltus, A., Skaletsky, H., Royce-Tolland, M., Dewar, K., and Page, D.C. (2004). An X-to-autosome retrogene is required for spermatogenesis in mice. Nat. Genet. *36*, 872–876.
14. Khil, P.P., Smirnova, N.A., Romanienko, P.J., and Camerini-Otero, R.D. (2004). The mouse X chromosome is enriched for sex-biased genes not subject to selection by meiotic sex chromosome inactivation. Nat. Genet. *36*, 642–646.
15. Banks, K.G., Johnson, K.A., Lerner, C.P., Mahaffey, C.L., Bronson, R.T., and Simpson, E.M. (2003). Retroposon compensatory mechanism hypothesis not supported: Zfa knockout mice are fertile. Genomics *82*, 254–260.
16. Wu, C.I., and Xu, E.Y. (2003). Sexual antagonism and X inactivation–the SAXI hypothesis. Trends Genet. *19*, 243–247.
17. Jiang, L., Li, T., Zhang, X., Zhang, B., Yu, C., Li, Y., Fan, S., Jiang, X., Khan, T., Hao, Q., *et al*. (2017). RPL10L is required for male meiotic division by compensating for RPL10 during meiotic sex chromosome inactivation in mice. Curr. Biol. *27*, 1498–1505.e6.
18. Tan, S., Cardoso-Moreira, M., Shi, W., Zhang, D., Huang, J., Mao, Y., Jia, H., Zhang, Y., Liu, Z., Huang, X., *et al*. (2016). LTR-mediated retroposition as a mechanism of RNA-based duplication in metazoans. Genome Res. *26*, 1663–1675.
19. Wei, W., Gilbert, N., Ooi, S.L., Lawler, J.F., Ostertag, E.M., Kazazian, H.H., Boeke, J.D., and Moran, J.V. (2001). Human L1 retrotransposition: cis preference versus trans complementation. Mol. Cell Biol. *21*, 1429–1439.
20. Li, W.-H. (1997). Molecular Evolution (Sunderland: Sinauer).

Gene Evolution: Getting Something from Nothing

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http://dx.doi.org/10.1016/j.cub.2017.05.056

New genes arise from pre-existing genes, but some *de novo* origin from non-genic sequence also seems plausible. A new study has surprisingly concluded that 25% of random DNA sequences yield beneficial products when expressed in bacteria.

> *The probability that a functional protein would appear de novo by a random association of amino acids is practically zero.... creation of entirely new nucleotide sequences could not be of any importance in the production of new information.*
>
> — François Jacob [1]

When François Jacob wrote that "Nature is a tinkerer, not an inventor" in a famous essay [1], his point was that evolution works only with material immediately at hand, not with the foresight of an engineer. He emphasized the role of gene duplication in creating new genes with new functions; while doing so, he strongly