Signs of Genomic Battles in Mouse Sex Chromosomes

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http://dx.doi.org/10.1016/j.cell.2014.10.036

Y chromosomes are challenged by a lack of recombination and are transmitted to the next generation only via males. Sequencing of the mouse Y reveals how these properties drive opposing evolutionary processes: massive decay of ancestral genes and convergent acquisition and amplification of spermatid-expressed gene families on the X and Y chromosome. The convergent acquisition and amplification of X-linked paralogs on the Y maintains a surprisingly gene-rich, euchromatic mammalian male chromosome.

Sex chromosomes evolved independently many times from autosomes in different lineages; for example, the mammalian X and Y originated ~150 million years ago (Bellott and Page, 2009). Two features set sex chromosomes apart from the rest of the genome and drive their unique evolution. These are a lack of recombination on the male-specific region on the Y (MSY) and sex-biased or sex-limited transmission. The lack of recombination renders natural selection inefficient on the Y, resulting in the accumulation of detrimental mutations and, in the long term, massive gene decay (i.e., Y chromosome degeneration). Indeed, old Y chromosomes have lost most of their ancestral genes in many taxa, are often highly repetitive, and have become partly or fully heterochromatic. Sex-biased transmission of sex chromosomes, on the other hand, makes the X and Y chromosome a battleground for sexual conflict. This can result in the accumulation of mutations with sex-specific fitness effects and the invasion of selfish elements on sex chromosomes that cheat meiosis to gain preferential transmission at the expense of homologous loci and bias the sex ratio of progeny. Skewed population sex ratios strongly favor suppressor genes that re-establish fair meiosis and an equal sex ratio.

In this issue of *Cell*, Sho et al. (2014) present the genome sequence of the mouse Y chromosome, which bears the signatures of both a lack of recombination and genomic conflict between sex chromosomes to an extreme extent. Sequencing of Y chromosomes is challenging due to their high content of repetitive sequences. David Page and colleagues have devised a labor-intensive and careful strategy, termed single-haplotype iterative mapping and sequencing (SHIMS), to assemble ampliconic and highly repetitive sequences. This strategy was successfully employed by Page's lab to sequence several primate sex chromosomes (Bellott and Page, 2009), but none of the previously sequenced Y's provided such a technical challenge as the mouse Y due to its highly repetitive nature.

In contrast to the classical view of Y chromosomes being heterochromatic and gene poor, the mouse MSY is almost entirely euchromatic and contains about 700 protein-coding genes. The mouse Y chromosome consists of two sequence classes: ancestral sequence, which originated from the autosomal ancestor of the mammalian sex chromosomes, and acquired sequence not originally present on the ancestral autosomes. Ancestral sequence occupies only 2 Mb and is entirely located within the short arm of the chromosome. Its evolutionary history follows the classical trajectory of Y chromosome degeneration. Of 639 genes present on the ancestral autosomes, only 9 remain on the mouse Y, which is fewer than on other mammalian Y chromosomes (Bellott et al., 2014). Thus, the mouse MSY has experienced greater degeneration than the MSY of other mammals studied to date. The remaining, acquired sequence consists almost entirely of repeated sequences on the chromosome's long arm. The ampliconic sequence is made up of ~200 copies of a half-megabase unit that contains three rodent-specific protein-coding gene families. Members of the gene families are consequently massively amplified on the mouse Y (132, 197, and 317 copies of *Sly*, *Srsy*, *Ssty*, respectively, with intact ORFs), and their sequences are highly similar to each other. Ampliconic sequences have also been found to a lesser extent on the Y chromosome of other primates, including humans, and are thought to be a Y-specific adaptation that allows for intrachromosomal recombination and gene conversion to retard Y degeneration (Rozen et al., 2003). Like most Y-linked genes, members of these gene families are highly transcribed in testis, and their high copy number may be needed for high levels of gene expression (Mueller et al., 2013).

However, several observations suggest that the picture of gene amplification on the Y is not that simple. Intriguingly, the same gene families found on the mouse Y have been convergently acquired and amplified on the X chromosome, and both the X and Y acquired and amplified genes are expressed predominantly in the male germline (Mueller et al., 2013). Because the X undergoes normal recombination in female meiosis,

accumulation of amplicons on the X is unlikely for the benefit of allowing gene conversion among gene family members. In addition, gene amplification of these gene families had comparatively little effect on transcript abundance among species that differ in their copy number of gene families (Ellis et al., 2011). What then would be driving the convergent coamplification of testis-expressed gene families on both the X and the Y?

Genomic conflict between the X and the Y chromosome during spermatogenesis to increase their transmission (i.e., meiotic drive) might be responsible for the observed expansion of sex-linked spermatid-expressed genes in mouse. Males with a partial deletion of the long arm of the Y produce offspring with a sex ratio skewed toward females, suggesting that this arm encodes a factor suppressing sex ratio distortion (Cocquet et al., 2009). Detailed molecular analysis of the *Sly* and closely related X-linked *Slx*/*Slxl1* gene family provides strong support for segregation distortion as a major force driving gene acquisition and amplification on the mouse X and Y. In particular, *Sly* is essential for normal spermatogenesis, and *Sly*-deficient male lab mice have reduced fertility, show widespread overexpression of X-linked postmeiotic genes, including *Slx*/*Slxl1*, and produce female-biased litters (Cocquet et al., 2009). In contrast, deficiency of *Slx*/*Slxl1* reduces male fertility and results in male-biased litters (Cocquet et al., 2012), and simultaneous knockdown of all three genes significantly improves fertility, returns expression of sex-linked genes to wild-type levels, and restores equal sex ratio (Cocquet et al., 2012). These antagonistic interactions suggest that *Slx*/*Slxl1* and *Sly* are involved in an intragenomic conflict, and an evolutionary arms race between segregation distorter and repressor may drive their dramatic copy number expansion and perhaps amplification of several other X- and Y-linked genes in mice (Figure 1A). The phylogenetic pattern of *Slx*/*Slxl1* and *Sly* amplification is consistent with this meiotic drive scenario, i.e., they were first convergently acquired on sex chromosomes in the Palaearctic mouse clade, and closely related species differ dramatically in copy number (Ellis et al., 2011).

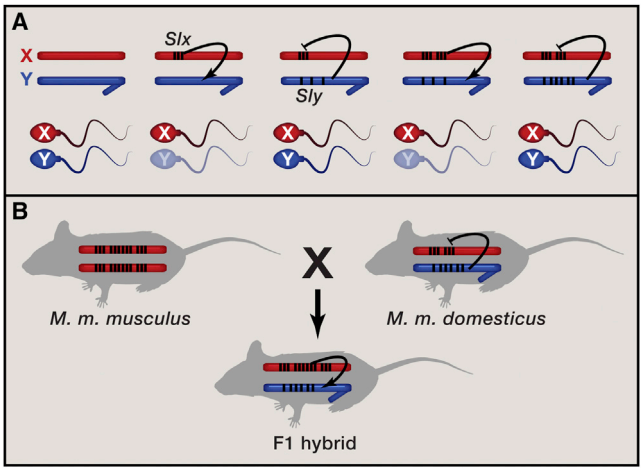

Figure 1. Why Convergent Acquisition and Amplification of Genes on Mouse X and Y Chromosomes May Occur, Yielding a Gene-Rich Euchromatic Mammalian Y Chromosome

(A) Model of recurrent bouts of coevolution between sex ratio distorters and suppressors. A sex ratio distorter on the X chromosome (*Slx*) that incapacitates Y-bearing sperm (indicated by arrow and transparent sperm) invades the population, skewing the population sex ratio. This creates a selective advantage to evolve a Y-linked suppressor (*Sly*) that is resistant to the distorter (indicated by repression line). Increased copy number of *Slx* can increase its ability to drive against the Y chromosome, and the Y will respond by increasing the copy number of *Sly* to neutralize the effects of increased *Slx*-dose.

(B) Coevolution of sex ratio distorters and suppressors may contribute to F1 male hybrid sterility in crosses between *M. m. musculus* mothers and *M. m. domesticus* fathers, which differ in their copy number *for Slx*/*Slxl1* and *Sly* genes.

The occurrence of sex ratio distortion in a population can often be transient and can easily escape notice, but recurrent bouts of sex ratio meiotic drive and its subsequent suppression can have important consequences on sex chromosome biology and speciation (Meiklejohn and Tao, 2010). For example, they can lead to incompatibilities that cause sterility in hybrids and thus might contribute to reproductive isolation between species. Intriguingly, F1 hybrid sterile males produced by asymmetric crosses between *M. m. musculus* mothers and *M. m. domesticus* fathers display sperm differentiation defects and widespread overexpression of X-linked genes, mimicking the phenotype of *Sly*-deficient mice (Good et al., 2010); males from the reciprocal cross are fertile. Sterile F1 males with a *M. m. musculus* X chromosome and *M. m. domesticus* Y have an excess of *Slx*/*Slxl1* copies compared to *Sly* copies (i.e., estimated copy number for *Slx*/*Slxl1* and *Sly* on the X and Y are about 50 copies for *M. m. domesticus* and about 100 copies for *M. m. musculus*; Ellis et al., 2011), and the relative deficiency in the number of *Sly* copies may contribute to F1 male hybrid sterility (Figure 1B) and could explain the observed overexpression of X-encoded genes and associated sperm defects and infertility.

Having the full mouse Y chromosome sequence in hand will aid in the molecular dissection of function of other gene families on the mouse sex chromosomes, and further sequencing of other species' sex chromosomes should allow reconstruction of their evolutionary history. Detailed molecular and evolutionary analysis should shed light on the role that genetic conflict over sex chromosome transmission has played to shape the evolution of sex chromosome gene content and epigenetic regulation, as well as its contribution to hybrid sterility between species.

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Synthetic Biology Looks Good on Paper

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http://dx.doi.org/10.1016/j.cell.2014.10.003

Tremendous progress has been made in the design and implementation of synthetic gene circuits, but real-world applications of such circuits have been limited. Cell-free circuits embedded on paper developed by Pardee et al. promise to deliver specific and rapid diagnostics on a low-cost, highly scalable platform.

In an age of rapid biomedical advancements and during a push to move from bench to bedside, how can synthetic biology keep up with the expanding clinical need for high-throughput, accurate, and translational point-of-care diagnostics? Pardee and colleagues have a simple answer: paper (Pardee et al., 2014).

Over the last 15 years, numerous gene circuits have been engineered to program behaviors of individual cells or cell populations. In addition to addressing fundamental biological questions (Riccione et al., 2012; Cheng and Lu, 2012), these circuits and the lessons learned from designing them could have practical applications in medicine and biotechnology (Church et al., 2014). Despite impressive progress, however, our ability to program cellular behavior in a predictable manner remains limited. Furthermore, the use of engineered living cells for therapeutic applications faces substantial challenges in overcoming public safety concerns and regulatory hurdles.

These challenges can be partially overcome by cell-free systems (Hockenberry and Jewett, 2012). These platforms consist of either purified gene expression machinery or cell extracts, an energy source (Sun et al., 2014), and the desired DNA constructs of interest. Bypassing the need to deal with complex cellular dynamics allows cell-free systems to be used for rapid iterative prototyping of gene circuits. Also, cell-free systems provide a more controlled environment, which can act as a powerful foundation for increasingly complex synthetic biology applications, such as biosensors. However, solution-based reactions of typical cell-free systems must follow strict protocols such as proper freeze-thaw, temperature regulation, and sample preparation to achieve reproducible results. To overcome these difficulties, Pardee and colleagues demonstrate how paper can be used as a vehicle to embed, store, distribute, and operate diverse gene circuits in a robust manner (Pardee et al., 2014), which lays the foundation for low-cost yet accurate diagnostics.

Their key innovation is to embed the gene expression machinery along with well-defined gene circuits in filter paper by freeze-drying. Doing so renders the operation of gene circuits less constrained by typical laboratory conditions and the complexity of cellular environments. The methods used to reconstitute the reactions from paper and run the experiments involve only a few simple steps. Using this platform, the authors demonstrate the storage and operation of a wide variety of gene circuits. These span constitutive and inducible gene expression, a set of sophisticated RNA-based circuits (toehold switches), and gene expression cascades (Figure 1A). In fact, engineering of the toehold circuits represents a major innovation in its own right (Green et al., 2014 in this issue of *Cell*). As with riboregulators, toehold switches contain a transducer RNA with its ribosome-binding site (RBS) sequestered in a hairpin, preventing target gene expression. Gene expression is activated by a trigger RNA that exposes the RBS by unwinding the hairpin. However, in the toehold switch, the RBS is moved to the loop region of the hairpin, allowing the trigger RNA to take virtually any sequence. This design greatly expands the diversity of target sequences that RNA circuits can sense. Indeed, these