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Sex chromosome evolution: life, death and repetitive DNA

Nikita Deshpande and Victoria H Meller*
Department of Biological Sciences; Wayne State University; Detroit, MI USA

Dimorphic sex chromosomes create problems. Males of many species, including *Drosophila*, are heterogametic, with dissimilar X and Y chromosomes. The essential process of dosage compensation modulates the expression of X-linked genes in one sex to maintain a constant ratio of X to autosomal expression. This involves the regulation of hundreds of dissimilar genes whose only shared property is chromosomal address. *Drosophila* males dosage compensate by up regulating X-linked genes 2 fold. This is achieved by the Male Specific Lethal (MSL) complex, which is recruited to genes on the X chromosome and modifies chromatin to increase expression. How the MSL complex is restricted to X-linked genes remains unknown. Recent studies of sex chromosome evolution have identified a central role for 2 types of repetitive elements in X recognition. Helitrons carrying sites that recruit the MSL complex have expanded across the X chromosome in at least one Drosophila species.[1] Our laboratory found that siRNA from an X-linked satellite repeat promotes X recognition by a yet unknown mechanism.[2] The recurring adoption of repetitive elements as X-identify elements suggests that the large and mysterious fraction of the genome called "junk" DNA is actually instrumental in the evolution of sex chromosomes.

Keywords: dosage compensation, helitrons, sex chromosomes, satellite repeats, 1.688^{X} repeats, 359 bp repeats

*Correspondence to: Victoria H Meller; Email: vmeller@biology.biosci.wayne.edu

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Many eukaryotes determine sex with dimorphic sex chromosomes, such as X and Y. Y chromosomes have dramatically diminished coding potential, and this produces problems for the organisms that carry them.[3] Recombination between the X and Y produces abnormal chromosomes, and must therefore be suppressed in the male germ line. In addition, the somatic expression of X-linked genes must be adjusted so that males and females have equivalent levels of most proteins encoded on the X. Mechanisms that recognize and modulate expression from the X chromosome, termed dosage compensation, have arisen numerous times.[4] The diverse epigenetic machinery that has been recruited for this purpose is the subject of many excellent reviews. But systems of compensation share something remarkable and less well understood: the ability to coordinate modulation of nearly all the genes on a single chromosome. We use an evolutionary perspective to argue that mobile elements and repetitive DNA are determinants of X chromosome identity in flies. New studies from our lab and others now implicate different types of repetitive DNA in recruitment of dosage compensation to the fly X chromosome. Interestingly, mobile elements are also a destructive force in sex chromosome evolution. The non-recombining Y chromosomes are havens for mobile DNA, leading to rapid erosion of coding potential.[5] The duality of these roles suggests that repetitive sequences underlie the evolutionary plasticity of fly sex chromosomes.

D. melanogaster, and related species, achieves dosage compensation by increasing transcription from the male X chromosome approximately 2-fold. This occurs by selective recruitment of a ribonucleoprotein complex, the Male Specific Lethal (MSL) complex, to transcribed X-linked genes.[6] The MSL complex acetylates H4 on lysine 16 (H4K16Ac), a modification that facilitates transcriptional elongation, and possibly initiation.[7,8] While the action of the MSL complex on chromatin is well studied, what limits the complex to the X chromosome remains unclear. A group of X-linked sites termed

Chromatin Entry Sites (CES) recruits the MSL complex, which then moves into nearby transcribed genes.[9] CES contain a 21 bp MSL Recognition Element (MRE) that binds a protein called CLAMP.[10] Knock down of CLAMP blocks X chromosome binding of MSL proteins, demonstrating its importance for X recognition. However, MREs are only modestly enriched on the X chromosome. Furthermore, CLAMP binds autosomal MREs but fails to recruit MSL proteins to autosomal sites. The question of what enables the MSL complex to selectively bind X chromatin remains an open question.

Comparative studies of repetitive DNA in the *Drosophila* species group reveals enrichment of different types of repetitive DNA on the X chromosome, and this occurs in parallel to the acquisition of dosage compensation. *D. miranda* provides a fascinating model as it has 3 X chromosomes of different ages and uses MREs to attract the MSL complex.[11] The youngest X chromosomes were produced by fusions between autosomes and sex chromosomes.[12] Orthologous to the *D. melanogaster* X is the *D. miranda* XL, over 60 million years old.[13] The *D. miranda* XR is 15 million years old, and the neo-X chromosome is 1 million years old.[14] The neo-X chromosome of *D. miranda* is in the process of acquiring MREs and enrichment for H4K16Ac in males, but this process is near-complete on the XR.[1,15] Astonishingly, half of existing MREs on the neo-X are found in a transposable element called ISX.[1] ISX arose by mutation of an existing helitron, and subsequent expansion of this element on the neo-X. Furthermore, some MREs on the older XR originated from a different helitron, ISXR, which also suffered a mutation that enabled MSL complex recruitment. While this is compelling, the example of *D. melanogaster* suggests that MREs are not the sole element that ensures selective recruitment of dosage compensation.

Our laboratory previously demonstrated that mutations in the siRNA (small interfering RNA) pathway are potent enhancers of mutations that impair X recognition during dosage compensation in *D. melanogaster* males.[16] This was exciting because many organisms modify chromatin using the siRNA pathway. In brief, double stranded RNA from bidirectional transcription is processed into siRNA. siRNA associates with Argonaute proteins, which in turn guide chromatin-modifying complexes to nascent RNAs with identity to the siRNA.[17] However, no physical interactions between the MSL complex and components of the siRNA pathway have been discovered, suggesting an indirect mode of action. As many repetitive sequences are transcribed from both strands, these became candidates for the source of chromosome-specific siRNAs.

Our attention was attracted by a family of satellite repeats that is near-exclusive to the *D. melanogaster* X chromosome and produces siRNA. The 1.688 g/cm^3 repeats (1.688^X repeats) are dispersed throughout X euchromatin in short, tandem clusters.[2] Unusual for repetitive elements, 1.688^X repeats are enriched in active regions, often in introns.[18] This inspired the suggestion that the 1.688^X repeats could serve to modulate expression. Examination of chromosome-specific repeats in several species revealed that X chromosome enrichment for repetitive satellites is strikingly conserved in *Drosophila* species, even when the precise sequence of these repeats is not.[2,19] Furthermore, the neo-X chromosome of *D. pseudoobscura* (similar to the XR chromosome of *D. miranda*) has acquired 1.688^X repeats, but the autosomes are devoid of them.[19] Repeats that are limited to the X chromosome thus coincides with the origin of new X chromosomes.

Could the *D. melanogaster* 1.688^X repeats produce a chromosome-specific siRNA that helps identify X chromatin? To address this question, long single stranded RNA and double stranded RNA was ectopically expressed in flies with moderately reduced male survival due to impaired X recognition. Single stranded 1.688^X RNA further reduced male survival, but double stranded RNA from one 1.688^X repeat dramatically rescued males and partially restored MSL localization to the X-chromosome.[2] Based on this, we put forth a model in which siRNA produced from 1.688^X repeats serves to recruit potential chromatin modifiers to similar X-linked regions. Rather than recruiting the MSL complex directly, we postulate that alteration of chromatin at 1.688^X repeats allows the X chromosome to assume a characteristic interphase conformation that facilitates recognition, or distribution of the MSL complex along the chromosome. In support of this idea, the X chromosome assumes different conformations in the interphase nuclei of males and females.[20] Although our studies focused on *Drosophila*, one of the major classes of mammalian repetitive DNA has long been suspected to play a role in dosage compensation. Mammals dosage compensate by inactivating a single X chromosome in females.[21] Long Interspersed Nuclear Elements 1 (L1 elements) are enriched on the mammalian X and have been proposed to assist recognition of X chromatin, or spreading of silencing, during X-inactivation in mammals.[22] Interestingly, the formation of the inactive X territory during early differentiation is coincident with a burst of siRNA production by the L1 elements.[23] We postulate that in both flies and mammals the challenge of selectively recognizing an entire chromosome is met with a combination of collaborating epigenetic pathways, one of which relies on small RNA produced from the X chromosome.

These findings raise several intriguing questions. Do X-enriched satellite repeats in other *Drosophila* species produce siRNA that promotes X recognition? If so, the rapid turnover of these repeats may be a factor in hybrid incompatibilities, which preferentially effect males, sometimes disrupting dosage compensation.[24] Interestingly, over 10 Mb of pericentric X heterochromatin is composed of similar 1.688^X repeats in *D. melanogaster*, but not in related species.[25] When hybrid matings introduce the *D. melanogaster* X chromosome into *D. simulans* ooplasm, the heterochromatin of the *D. melanogaster* X fails to resolve during early mitotic divisions, causing hybrid female lethality.[26] One possible explanation is that *D. simulans* oocytes lack abundant 1.688^X small RNAs that are present in *D. melanogaster* and may be necessary to initiate formation of heterochromatin at the 1.688^X repeats. Consistent with these ideas, removal of nearly all *D. melanogaster* X heterochromatin by the *Zhr*[1] translocation rescues

mitosis in hybrid females.[26] These studies suggest that a single, rapidly evolving class of repetitive sequences on the fly X chromosome intersects with sex chromosome biology in ways that critically influence viability and reproduction.

Eukaryotic genomes are rich with repetitive elements, often referred to as junk DNA, that have few known functions. Recent studies reveal that chromosome-specific repetitive elements and small RNA-based chromatin regulation have been repeatedly adapted to guide epigenetic regulation of a chromosome. The ability to direct dosage compensation to an entire linkage group is an essential step in the evolution of dimorphic sex chromosomes. As repetitive sequences are also implicated in hybrid incompatibilities, we postulate that they confer "evolvability" upon the predecessors of highly differentiated sex chromosomes, and also contribute to the development of species.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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