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Identification of the Drosophila X chromosome: The long and short of it

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The different dose of X chromosomes in males and females produces a potentially fatal imbalance in X-linked gene products. This imbalance is addressed by dosage compensation, a process that modulates expression from an entire X chromosome in one sex. Dosage compensation acts on thousands of genes with disparate expression patterns. Both flies and mammals accomplish this with remarkable specificity by targeting epigenetic chromatin modifications to a single chromosome. Long noncoding RNAs that are expressed from the X chromosome are essential elements of the targeting mechanism in both lineages. We recently discovered that the siRNA pathway, as well as small RNA from satellite repeats that are strikingly enriched on the fly X chromosome, also promote X recognition. In this article we review the current understanding of X recognition in flies and discuss potential mechanisms by which the siRNA pathway, repetitive elements and long noncoding RNAs might cooperate to promote X recognition.

Keywords: 1.688^{X} repeats, 359 bp repeats, dosage compensation, Drosophila melanogaster, roX RNA, siRNA, satellite repeats, X recognition

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Introduction

Sex chromosomes produce genetic imbalance

Although the primary signals that direct sexual development are remarkably varied, the adaptation of a pair of chromosomes to control this process is common. Organisms as diverse as flies and humans have XY males and XX females. The emergence of dimorphic sex chromosomes is driven by degradation of the Y chromosome in response to transmission solely through one sex.[1] In consequence, the Y chromosomes of flies and humans, which are of completely different origin, are both gene poor and largely heterochromatic. In contrast, the X chromosomes are gene rich. Heterogametic sex chromosomes therefore produce an imbalance in X-linked gene dosage between males and females. This imbalance is addressed early in development by a process known as dosage compensation.[2]

Chromosome-wide modulation of gene expression

Maintenance of a balanced X to autosome gene expression ratio is essential in flies and mammals. Eutherian mammals accomplish this by a global increase of X-linked gene expression that restores the X to autosome ratio in males.[3] Females accommodate increased expression by inactivation of a single, randomly selected X chromosome in every cell during early embryogenesis.[2] Inactivation is initiated by expression of the *X inactive specific transcript* (*Xist*) from one of the 2 X chromosomes in female cells.[4] *Xist*, a long noncoding RNA, recruits complexes that modify and silence chromatin. Silencing spreads in *cis* to inactivate a single X chromosome. Additional features of the X chromosome, such as interphase organization and repetitive elements on the X, facilitate recognition and silencing.[4,5]

Flies also increase the expression of X-linked genes, but through a mechanism that is limited to males. Almost all genes on the single X chromosome of males are bound by a complex of proteins and RNA that modifies chromatin to increase expression.[6] This complex, known as the Male Specific Lethal (MSL) complex, is composed of 5 MSL proteins and one of 2 non-coding RNA on the X RNAs (*roX1, roX2*). Females block expression of a key MSL protein, and this limits X upregulation to males.[7] The MSL complex is recruited to transcribed genes.[8] A histone

acetyltransferase in the complex, Males absent on the first (MOF), places H4Ac16 marks at sites of MSL complex binding.[9,10] The precise molecular mechanism by which the MSL complex elevates expression remains a topic of debate. The H4K16Ac mark partially decondenses chromatin and increases transcriptional elongation at X-linked genes.[11-14] Compensation may also involve a modest enrichment of RNA polymerase II (RNAP II) at promoters, and the mammalian homolog of one complex member is an E3 ubiquitin ligase that modifies H2B and promotes early transcriptional elongation.[15-17] Regardless of the transcriptional steps that are modulated to achieve elevated expression, association of the MSL complex with the male X chromosome is remarkably specific, limiting this process to X-linked genes.

Identification of X chromatin is a multi-step process

Elegant studies by several groups have established a sequential model for recruitment of the MSL complex to X chromatin. A number of X-linked Chromatin Entry Sites (CES) were initially characterized by their robust recruitment of remaining MSL proteins in males mutated for some members of the complex.[18] A key feature of CES is the MSL Recognition Element (MRE), a 21 bp sequence that is modestly enriched on the X chromosome.[19,20] An RNAi screen designed to find genes required for X-localization identified CLAMP, a zinc finger protein that binds MREs and is essential for MSL recruitment.[21] Although CLAMP is necessary for compensation, it also binds to autosomal MREs that do not recruit the MSL complex. Furthermore, CLAMP is an essential gene in both sexes, indicating a role outside of dosage compensation. After recruitment by CES the MSL complex spreads into nearby transcribed genes through binding of one complex member, Male Specific Lethal 3 (MSL3) to the cotranscriptional H3K36me3 mark.[22-24] In accord with this the Set2 methyltransferase, responsible for H3K36me3 deposition, is essential for spreading of the complex from CES into nearby genes.[23,25] These studies were used to formulate a well-supported model of local recruitment in which initial binding to CES is followed by spreading into active genes.[26] However, both recruitment to the CES and subsequent spreading into nearby genes rely on features that are not specific to the X chromosome. How X chromatin is recognized with sufficient specificity remains an open question. We recently demonstrated that small RNA contributes to X recognition.[27] In this article we propose mechanisms by which small RNAs might act to ensure efficient X recognition by the MSL complex.

The roX genes play multiple roles in MSL recruitment to the X chromosome

RNA produced by *roX1* and *roX2* assembles with the MSL proteins and can be visualized along the length of the male X chromosome.[28] Mutation of a single *roX* gene has no obvious phenotype, but simultaneous loss of *roX1* and *roX2* results in mislocalization of MSL proteins to ectopic autosomal sites.[29] Reduced expression of X-linked genes in *roX1 roX2* males leads to lethality.[30] The *roX* genes have unusual properties that may contribute to X recognition. Both *roX* genes are X-linked and overlap CES. In accordance with this, the *roX* genes themselves recruit the MSL complex and promote spreading into flanking chromatin.[31] Remarkably, autosomal insertions of *roX* transgenes recruit MSL proteins, which then spread into autosomal chromatin flanking the transgene.[32,33] The ability of *roX* to recruit modifying activities to chromatin in *cis* is reminiscent of *Xist*, and undoubtedly contributes to normal X recognition in flies. However, *roX* RNA from autosomal transgenes also assembles with the MSL proteins and travels to the X chromosome, restoring compensation and rescuing *roX1 roX2* males.[29] Although *roX* RNA is essential for X recognition, the location of both *roX* genes on the X chromosome is thus not essential for identification of X chromatin.

The siRNA pathway contributes to X recognition

A clue to how X chromatin is recognized lies in the discovery that the siRNA pathway contributes to this process.[27] Loss of the siRNA binding protein Argonaut 2 (Ago2) has little effect on MSL localization or viability in otherwise normal males, but is almost completely male-lethal in weakly hypomorphic *roX1 roX2* flies. Lethality is accompanied by severe disruption of MSL localization, which is not otherwise observed in this particular *roX1 roX2* mutant. Several additional genes that are necessary for production of siRNA also interact genetically with *roX1 roX2* mutants.[27] This raises intriguing questions, such as the nature of small RNAs involved and the mechanism by which this pathway contributes to X chromosome recognition. Small RNA pathways have been shown to modulate chromatin and gene expression in flies, but typically act to repress expression or promote heterochromatin formation. For example, the Piwi pathway, responsible for germ line silencing of mobile elements, and the siRNA pathway have both been implicated in formation of heterochromatin in somatic tissues.[34-37] Ago2 binds chromatin and is involved in transcriptional repression of some genes.[38] It is possible that repression, at least in some instances, is mechanistically similar to that in *Schizosaccharomyces pombe*, where nascent RNAs are bound by siRNA-containing effector complexes that recruit epigenetic modifiers.[39,40]

Although the involvement of the siRNA pathway in X recognition is intriguing, a possible mode of action is not immediately clear. First, there is no evidence that the MSL complex interacts directly with proteins of the siRNA system, even in the chromatin-bound context.[41] An additional complication is that the MSL complex deposits activating marks, but the siRNA pathway is typically associated with repression.[42] These considerations suggest that the siRNA pathway could modulate MSL complex localization through an indirect mechanism. Speculation about how small RNA contributes to X recognition was inspired by the identification of candidate siRNAs.

A family of X-linked repeats

The discovery that the siRNA pathway contributes to X recognition prompted a search for the source of the small RNA involved. Obvious candidates are small RNAs produced from a family of satellite repeats with remarkable enrichment on

the X chromosome.[43] These comprise a large block of pericentric heterochromatin, and related repeats are distributed in short, tandem clusters throughout X euchromatin. These AT-rich sequences are variously known as the 359 bp (repeating unit), or 1.688 g/cm^3 (buoyant density in cesium chloride) satellites. We have adopted the 1.688^X designation for the euchromatic repeats to reflect a physical property and genomic location. The remarkable distribution of the 1.688^X satellites has long prompted speculation that they might participate in dosage compensation.[44] In *Drosophila melanogaster* many 1.688^X satellites are transcribed from both strands, and small RNA from these repeats is detectable in some fly tissues.[45,46] Unlike most repetitive DNA, the euchromatic 1.688^X satellites are often near or within genes.[47] Several properties of the 1.688^X satellites suggest functionality. First, enrichment for satellite repeats on the X chromosome is conserved in related species, even if the sequence of these repeats is not.[48] Strikingly, a neo-X chromosome produced by an X and autosome fusion has been rapidly invaded by satellite repeats.[48]

siRNA from one 1.688^X repeat promotes X recognition

The large number and dispersed localization of 1.688^X repeats on the X precludes functional testing by deletion. Instead, we decided to test whether ectopic production of short RNA from selected repeat clusters was biologically active in flies. On average, X-linked 1.688^X repeat family members share 73% sequence identity.[47] We selected 3 clusters, designated by superscripts indicating cytological position. One is immediately distal to *roX1* (1.688^{3F}), one is at the tip of the X chromosome (1.688^{1A}, 89% identity to 1.688^{3F}), and a third is situated between these (1.688^{3C}, 69% identity to 1.688^{3F}). Ectopic production of double stranded hairpin RNAs from these repeats, which are readily processed into siRNA, had no apparent effect on otherwise wild type males. We then tested the survival of males with partial to complete loss of *roX1 roX2* function. Double stranded RNA from 1.688^{1A} or 1.688^{3C} had little or no effect, but double stranded RNA from 1.688^{3F} dramatically rescued male survival.[45] Amazingly, recovery of adult males with a *roX1 roX2* chromosome that supports fewer than 1% adult escapers was increased to over 30%! In parallel with increased survival, localization of the MSL proteins to the X chromosome was partially restored in these males. It is particularly intriguing that only siRNA from 1.688^{3F} was capable of rescuing *roX1 roX2* males, as the regions tested share considerable similarity.[45] The 1.688^{3F} repeat cluster is located just a few hundred bases distal to *roX1*. Interestingly, *roX1* is expressed several hours earlier than *roX2* and is solely responsible for initial X recognition.[49] The adjacent location of 2 elements involved in X recognition suggests spatial integration of cooperating pathways.

The discovery of siRNAs that promote compensation is exiting, but interpretation of these findings is far from obvious. Dosage compensation and siRNA-dependent epigenetic processes both alter chromatin structure. Although it is unlikely that the siRNA pathway directly recruits the MSL complex, genetic interactions between siRNA and *roX1 roX2* mutants suggests that both participate in X-recognition. One possibility is that the siRNA pathway modifies chromatin at the 1.688^X repeats, and this change promotes X recognition.

Do 1.688^X repeats enhance transcription of nearby genes?

One possibility is that the 1.688^X repeats act as enhancers to facilitate transcription of X-linked genes, which in turn might impact MSL recruitment. The transcriptional status of compensated genes, as well as epigenetic factors associated with activation, are factors in MSL recruitment.[22,23,50] In support of this idea, repetitive elements have been adapted for gene regulation in other organisms. Recent studies in mammals describe the evolution of *Alu* repeats to acquire the chromatin features of poised and active enhancers (enrichment for H3K4me1 or H3K27ac and P300).[51] Transcripts originating from mammalian enhancer regions have been implicated in the recruitment of RNAP II to gene promoters.[52-54] We observe widespread transcription from the fly 1.688^X repeats, which could be related to enhancer function.[45] Enhancer activity might be modulated by siRNA-directed modification of 1.688^X repeats. Although usually implicated in repression, Ago2 has also been linked to activation in a few instances in flies and humans.[55-58] It would be interesting to determine whether epigenetic features of *Drosophila* enhancers are found at 1.688^X repeats.[59] In spite of the large number of genome-wide studies of chromatin modifications that have been performed in flies, highly repetitive sequences are typically removed from analysis because they are difficult or impossible to map.

Do 1.688^X repeats influence chromatin architecture or topology?

Features of the 1.688^X repeats suggest a potential role in modulation of chromosome architecture. The 1.688^X repeats are AT rich, a common feature of DNA that interacts with proteins of the nuclear matrix, such as satellite binding proteins and Topoisomerase II (Top2).[60] Top2, a major component of the nuclear matrix, has also been shown to bind pericentromeric 359 nt repeats in interphase nuclei.[61,62] Furthermore, Top2, and the associated DNA supercoiling factor SCF, both influence dosage compensation.[63,64] Top2 and SCF control DNA topology, suggesting that topological constraints on transcriptional initiation or elongation could be a factor in dosage compensation. For example, topological alterations might favor MSL complex association or spreading along the X chromosome.

Other aspects of nuclear organization may also be modulated by the 1.688^X repeats. The male X chromosome is reported to associate with the nuclear envelope.[65] Some nuclear pore proteins are enriched in regions of active expression, and these are particularly prominent on the male X chromosome, overlapping MSL-bound genes.[66] One possibility is that 1.688^X repeats mediate association of the X chromosome with the nuclear periphery. Long-range interactions between 1.688^X repeats at different locations on the X chromosome could also be instrumental in establishing a chromosome-specific interphase architecture. In support of this idea, the organization of

the X chromosome is discernably different in males and females, and compensated genes are closer together in the interphase male nucleus than non-compensated regions.[67] An intriguing possibility is that siRNA-directed chromatin modifications regulate long-range interactions between 1.688^{X} repeats, or between repeats and the nuclear envelope. If 1.688^{X} repeats do participate in long-range interactions, this would bring *roX1*, immediately adjacent to 1.688^{3F}, close to distant regions of the X chromosome with 1.688^{X} repeats, providing a mechanism for MSL complexes assembled at *roX1* to rapidly access distant regions of the X chromosome. The possibility of long range interactions between distant 1.688^{X} repeats could be tested by Chromosome Conformation Capture (3C), a molecular method to identify chromosome loops.[68]

Insulators are another potential link between nuclear organization and X chromosome dosage compensation.[69,70] Insulators regulate enhancer access to promoters and govern folding of the genome.[70] Examples of repeats that act as insulators include long tandem repeats that mark the heterochromatin/euchromatin boundary on the *Drosophila* X chromosome, the human *DXZ4* macrosatellite repeat that regulates the nuclear organization of the inactive X chromosome and the sub-telomeric *D4Z4* repeats that oppose position effects.[69,71-74] Interestingly, both RNA and RNAi proteins, including Ago2, are implicated in the function of fly insulators.[56,75] The capacity of 1.688^{X} repeats to act as insulators could be determined using a well-established genetic assay.[76]

Emerging functions of repetitive sequences

Repetitive sequences make up large portions of eukaryotic genomes, yet the known functions of these repeats are largely limited to their participation in chromosome compaction and segregation. Repeats are central to centromere formation, and rapidly evolving repeats are features of meiotic drive systems.[77] Indeed, sequences closely related to the euchromatic 1.688^{X} repeats have been implicated in both processes.[78,79] In contrast to these mitotic or meiotic roles, the ideas presented in this article highlight the potential for repetitive regions to act as regulators of gene expression in the interphase nucleus. The involvement of satellite repeats in X chromosome dosage compensation presents a powerful experimental system in which to dissect the regulatory potential of repetitive DNA.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Charlesworth B. The evolution of sex chromosomes. Science 1991; 251:1030-3; PMID:1998119; http://dx.doi.org/10.1126/science.1998119
2. Lucchesi J, Kelly W, Panning B. Chromatin remodeling in dosage compensation. Ann Rev Genet 2005; 39:615-51; http://dx.doi.org/10.1146/annurev.genet.39.073003.094210
3. Deng X, Hiatt JB, Nguyen DK, Ercan S, Sturgill D, Hillier LW, Schlesinger F, Davis CA, Reinke VJ, Gingeras TR, et al. Evidence for compensatory upregulation of exressed X-linked genes in mammals, Caenorhabditis elegans and Drosophila melanogaster. Nat Genet 2011; 43:1179-85; http://dx.doi.org/10.1038/ng.948
4. Hall LL, Lawrence JB. XIST RNA and architecture of the inactive X chromosome: implications for the repeat genome. Cold Spring Harb Symp Quant Biol 2010; 75:345-56; http://dx.doi.org/10.1101/sqb.2010.75.030
5. Leung KN, Panning B. X-inactivation: Xist RNA uses chromosome contacts to coat the X. Curr Biol 2014; 24:R80-2; PMID:24456982; http://dx.doi.org/10.1016/j.cub.2013.11.052
6. Kong Y, Meller VH. How to get extra performance from a chromosome: recognition and modification of the X chromosome in male Drosophila melanogaster. Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics: John Wiley & Sons, Ltd, 2007; 15
7. Kelley R, Solovyeva I, Lyman L, Richman R, Solovyev V, Kuroda M. Expression of MSL2 causes assembly of dosage compensation regulators on the X chromosomes and female lethality in Drosophila. Cell 1995; 81:867-77; PMID:7781064; http://dx.doi.org/10.1016/0092-8674(95)90007-1
8. Alekseyenko AA, Larschan E, Lai WR, Park PJ, Kuroda MI. High-resolution ChIP-chip analysis reveals that the Drosophila MSL complex selectively identifies active genes on the male X chromosome. Genes Dev 2006; 20:848-57; PMID:16547173; http://dx.doi.org/10.1101/gad.1400206
9. Akhtar A, Becker P. Activation of transcription through histone H4 acetylation by MOF, and acetyltransferase essential for dosage compensation in Drosophila. Mol Cell 2000; 5:367-75; PMID:10882077; http://dx.doi.org/10.1016/S1097-2765(00)80431-1
10. Smith ER, Pannuti A, Gu W, Steurnagel A, Cook RJ, Allis CD, Lucchesi JC. The Drosophila MSL complex acetylates histone H4 at lysine 16, a chromatin modification linked to dosage compensation. Mol Cell Biol 2000; 20:312-18; http://dx.doi.org/10.1128/MCB.20.1.312-318.2000
11. Shogren-Knaak M, Ishii H, Sun J-M, Pazin MJ, Davie JR, Peterson CL. Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science 2006; 311:844-47; PMID:16469925; http://dx.doi.org/10.1126/science.1124000
12. Liu Y, Lu C, Yang Y, Fan Y, Yang R, Liu CF, Korolev N, Nordenskiold L. Influence of histone tails and H4 tail acetylations on nucleosome-nucleosome interactions. J Mol Biol 2011; 414:749-64; http://dx.doi.org/10.1016/j.jmb.2011.10.031
13. Larschan E, Bishop EP, Kharchenko PV, Core LJ, Lis JT, Park PJ, Kuroda MI. X chromosome dosage compensation via enhanced transcriptional elongation in Drosophila. Nature 2011; 471:115-8; PMID:21368835; http://dx.doi.org/10.1038/nature09757
14. Ferrari F, Plachetka A, Alekseyenko AA, Jung YL, Ozsolak F, Kharchenko PV, Park PJ, Kuroda MI. Jump start and gain model for dosage compensation in Drosophila based on direct sequencing of nascent transcripts. Cell Rep 2013; 5:629-36; PMID:24183666; http://dx.doi.org/10.1016/j.celrep.2013.09.037
15. Conrad T, Cavalli FMG, Vaquerizas JM, Luscombe NM, Akhtar A. Drosophila dosage compensation involves enhanced Pol II recruitment to male X-linked promoters. Science 2012; 337:742-46; PMID:22821985; http://dx.doi.org/10.1126/science.1221428
16. Wu L, Li L, Zhou B, Qin Z, Dou Y. H2B ubiquitylation promotes RNA Pol II processivity via PAF1 and pTEFb. Mol Cell 2014; 54:920-31; PMID:24837678; http://dx.doi.org/10.1016/j.molcel.2014.04.013
17. Wu L, Zee BM, Wang Y, Garcia BA, Dou Y. The RING finger protein MSL2 in the MOF complex is an E3 ubiquitin ligase for H2B K34 and is involved in crosstalk with H3 K4 and K79 methylation. Mol Cell 2011; 43:132-44; PMID:21726816; http://dx.doi.org/10.1016/j.molcel.2011.05.015
18. Lyman LM, Copps K, Rastelli L, Kelley RL, Kuroda MI. Drosophila Male-Specific Lethal 2 protein: structure/function analysis and dependence on MSL1 for chromosome association. Genetics 1997; 147:1743-53; PMID:9409833
19. Alekseyenko AA, Peng S, Larschan E, Gorchakov AA, Lee OK, Kharchenko P, McGrath SD, Wang CI, Mardis ER, Park PJ, et al. A sequence motif within chromatin entry sites directs MSL establishment on the Drosophila X chromosome. Cell 2008; 134:599-609; PMID:18724933; http://dx.doi.org/10.1016/j.cell.2008.06.033
20. Straub T, Grimaud C, Gilfillan GD, Mitterweger A, Becker PB. The chromosomal high-affinity binding sites for the Drosophila dosage compensation complex. PLoS Genet 2008; 4:e1000302; PMID:19079572; http://dx.doi.org/10.1371/journal.pgen.1000302
21. Soruco MM, Chery J, Bishop EP, Siggers T, Tolstorukov MY, Leydon AR, Sugden AU, Goebel K, Feng J, Xia P, et al. The CLAMP protein links the MSL complex to the X chromosome during Drosophila dosage compensation. Genes Dev 2013; 27:1551-6; PMID:23873939; http://dx.doi.org/10.1101/gad.214585.113
22. Kind J, Akhtar A. Cotranscriptional recruitment of the dosage compensation complex to X-linked target genes. Genes Dev 2007; 21:2030-40; PMID:17699750; http://dx.doi.org/10.1101/gad.430807
23. Larschan E, Alekseyenko AA, Gortchakov AA, Peng S, Li B, Yang P, Workman JL, Park PJ, Kuroda MI. MSL complex is attracted to genes marked by H3K36

trimethylation using a sequence-independent mechanism. Mol Cell 2007; 28:121-33; PMID:17936709; http://dx.doi.org/10.1016/j.molcel.2007.08.011
24. Sural TH, Peng S, Li B, Workman JL, Park PJ, Kuroda MI. The MSL3 chromodomain directs a key targeting step for dosage compensation of the Drosophila melanogaster X chromosome. Nat Struct Mol Biol 2008; 15:1318-25; PMID:19029895; http://dx.doi.org/10.1038/nsmb.1520
25. Bell O, Conrad T, Kind J, Wirbelauer C, Akhtar A, Schubeler D. Transcription-coupled methylation of histone H3 at lysine 36 regulates dosage compensation by enhancing recruitment of the MSL complex in Drosophila melanogaster. Mol Cell Biol 2008; 28:3401-9; PMID:18347056; http://dx.doi.org/10.1128/MCB.00006-08
26. Gelbart ME, Kuroda MI. Drosophila dosage compensation: a complex voyage to the X chromosome. Development 2009; 136:1399-410; PMID:19363150; http://dx.doi.org/10.1242/dev.029645
27. Menon DU, Meller VH. A role for siRNA in X-chromosome dosage compensation in Drosophila melanogaster. Genetics 2012; 191:1023-8; PMID:22554892; http://dx.doi.org/10.1534/genetics.112.140236
28. Meller VH, Wu KH, Roman G, Kuroda M, David R. roX1 RNA paints the X chromosome of male Drosophila and is regulated by the dosage compensation system. Cell 1997; 88:445-57; PMID:9038336; http://dx.doi.org/10.1016/S0092-8674(00)81885-1
29. Meller VH, Rattner B. The roX genes encode redundant male specific lethal transcripts required for targeting of the MSL complex. EMBO J 2002; 21:1084-91; PMID:11867536; http://dx.doi.org/10.1093/emboj/21.5.1084
30. Deng X, Meller VH. roX RNAs are required for increased expression of X-linked genes in Drosophila melanogaster males. Genetics 2006; 174:1859-66; PMID:17028315; http://dx.doi.org/10.1534/genetics.106.064568
31. Oh H, Park Y, Kuroda MI. Local spreading of MSL complexes from roX genes on the Drosophila X chromosome. Genes Dev 2003; 17:1334-9; PMID:12782651; http://dx.doi.org/10.1101/gad.1082003
32. Kageyama Y, Mengus G, Gilfillan G, Kennedy HG, Stuckenholz C, Kelley RL, Becker PB, Kuroda MI. Association and spreading of the Drosophila dosage compensation complex from a discrete roX1 chromatin entry site. EMBO J 2001; 20:2236-45; PMID:11331589; http://dx.doi.org/10.1093/emboj/20.9.2236
33. Kelley R, Meller VH, Gordadze P, Roman G, Davis R, Kuroda M. Epigenetic spreading of the Drosophila dosage compensation complex from roX RNA genes into flanking chromatin. Cell 1999; 98:513-22; PMID:10481915; http://dx.doi.org/10.1016/S0092-8674(00)81979-0
34. Fagegaltier D, Bouge AL, Berry B, Poisot E, Sismeiro O, Coppee JY, Theodore L, Voinnet O, Antoniewski C. The endogenous siRNA pathway is involved in heterochromatin formation in Drosophila. Proc Natl Acad Sci USA 2009; 106:21258-63; PMID:19948966; http://dx.doi.org/10.1073/pnas.0809208105
35. Brower-Toland B, Findley SD, Jiang L, Liu L, Yin H, Dus M, Zhou P, Elgin SCR, Lin H. Drosophila PIWI associates with chromatin and interacts directly with HP1a. Genes Dev 2007; 21:2300-11; PMID:17875665; http://dx.doi.org/10.1101/gad.1564307
36. Gu T, Elgin SC. Maternal depletion of Piwi, a component of the RNAi system, impacts heterochromatin formation in Drosophila. PLoS Genet 2013; 9:e1003780; PMID:24068954; http://dx.doi.org/10.1371/journal.pgen.1003780
37. Pal-Bhadra M, Leibovitch Ba, Gandhi SG, Rao M, Bhadra U, Birchler Ja, Elgin SCR. Heterochromatic silencing and HP1 localization in Drosophila are dependent on the RNAi machinery. Science 2004; 303:669-72; PMID:14752161; http://dx.doi.org/10.1126/science.1092653
38. Cernilogar FM, Onorati MC, Kothe GO, Burroughs AM, Parsi KM, Breiling A, Lo Sardo F, Saxena A, Miyoshi K, Siomi H, et al. Chromatin-associated RNA interference components contribute to transcriptional regulation in Drosophila. Nature 2011; 480:391-5; PMID:22056986; http://dx.doi.org/10.1038/nature10492
39. Buhler M, Verdel A, Moazed D. Tethering RITS to a nascent transcript initiates RNAi- and heterochromatin-dependent gene silencing. Cell 2006; 125:873-86; PMID:16751098; http://dx.doi.org/10.1016/j.cell.2006.04.025
40. Cam HP, Sugiyama T, Chen ES, Chen X, FitzGerald PC, Grewal SI. Comprehensive analysis of heterochromatin- and RNAi-mediated epigenetic control of the fission yeast genome. Nat Genet 2005; 37:809-19; PMID:15976807; http://dx.doi.org/10.1038/ng1602
41. Wang CI, Alekseyenko AA, LeRoy G, Elia AE, Gorchakov AA, Britton LM, Elledge SJ, Kharchenko PV, Garcia BA, Kuroda MI. Chromatin proteins captured by ChIP-mass spectrometry are linked to dosage compensation in Drosophila. Nat Structural Mol Biol 2013; 20:202-9; http://dx.doi.org/10.1038/nsmb.2477
42. Verdel A, Vavasseur A, Le Gorrec M, Touat-Todeschini L. Common themes in siRNA-mediated epigenetic silencing pathways. Int J Dev Biol 2009; 53:245-57; http://dx.doi.org/10.1387/ijdb.082691av
43. Kuhn GCS, Küttler H, Moreira-Filho O, Heslop-Harrison JS. The 1.688 repetitive DNA of drosophila: Concerted evolution at different genomic scales and association with genes. Mol Biol Evolution 2012; 29:7-11; http://dx.doi.org/10.1093/molbev/msr173
44. DiBartolomeis SM, Tartof KD, Jackson FR. A superfamily of Drosophila satellite related (SR) DNA repeats restricted to the X chromosome euchromatin. Nucleic Acids Res 1992; 20:1113-6; PMID:1549474; http://dx.doi.org/10.1093/nar/20.5.1113
45. Menon DU, Coarfa C, Xiao W, Gunaratne PH, Meller VH. siRNAs from an X-linked satellite repeat promote X-chromosome recognition in Drosophila melanogaster. Proc Natl Acad Sci USA 2014; 111:16460-5; PMID:25368194; http://dx.doi.org/10.1073/pnas.1410534111
46. Usakin L, Abad J, Vagin VV, de Pablos B, Villasante A, Gvozdev VA. Transcription of the 1.688 satellite DNA family is under the control of RNA interference machinery in Drosophila melanogaster ovaries. Genetics 2007; 176:1343-49; PMID:17409066; http://dx.doi.org/10.1534/genetics.107.071720
47. Kuhn G, Kuttler H, Moreira-Filho O, Heslop-Harrison J. The 1.688 repetitive DNA of Drosophila: concerted evolution at different genomic scales and association with genes. Mol Biol Evol 2011; 29(1):7-11
48. Gallach M. Recurrent turnover of chromosome-specific satellites in Drosophila. Genome Biol Evol 2014; 6:1279-86; http://dx.doi.org/10.1093/gbe/evu104
49. Meller VH. Initiation of dosage compensation in Drosophila embryos depends on expression of the roX RNAs. Mech Dev 2003; 120:759-67; http://dx.doi.org/10.1016/S0925-4773(03)00157-6
50. Wang CI, Alekseyenko Aa, LeRoy G, Elia AEH, Gorchakov Aa, Britton L-MP, Elledge SJ, Kharchenko PV, Garcia Ba, Kuroda MI. Chromatin proteins captured by ChIP-mass spectrometry are linked to dosage compensation in Drosophila. Nat Struct Mol Biol 2013; 20:202-9; http://dx.doi.org/10.1038/nsmb.2477
51. Su M, Han D, Boyd-Kirkup J, Yu X, Han JDJ. Evolution of Alu Elements toward Enhancers. Cell Rep 2014; 7:376-85; PMID:24703844; http://dx.doi.org/10.1016/j.celrep.2014.03.011
52. Johnson KD, Grass JA, Park C, Im H, Choi K, Bresnick EH. Highly restricted localization of RNA polymerase II within a locus control region of a tissue-specific chromatin domain. Mol Cell Biol 2003; 23:6484-93; PMID:12944475; http://dx.doi.org/10.1128/MCB.23.18.6484-6493.2003
53. Lam MTY, Li W, Rosenfeld MG, Glass CK. Enhancer RNAs and regulated transcriptional programs. Trends Biochem Sci 2014; 39:170-82; http://dx.doi.org/10.1016/j.tibs.2014.01.002
54. Mousavi K, Zare H, Dell'Orso S, Grontved L, Gutierrez-Cruz G, Derfoul A, Hager G, Sartorelli V. ERNAs Promote Transcription by Establishing Chromatin Accessibility at Defined Genomic Loci. Mol Cell 2013; 51:606-17; PMID:23993744; http://dx.doi.org/10.1016/j.molcel.2013.07.022
55. Cernilogar FM, Onorati MC, Kothe GO, Burroughs AM, Parsi KM, Breiling A, Sardo FL, Saxena A, Miyoshi K, Siomi H, et al. Chromatin-associated RNA interference components contribute to transcriptional regulation in Drosophila. Nature 2011; 480:391-5; PMID:22056986; http://dx.doi.org/10.1038/nature10492
56. Moshkovich N, Nisha P, Boyle PJ, Thompson BA, Dale RK, Lei EP. RNAi-independent role for Argonaute2 in CTCF/CP190 chromatin insulator function. Genes Dev 2011; 25:1686-701; PMID:21852534; http://dx.doi.org/10.1101/gad.16651211
57. Portnoy V, Huang V, Place RF, Li LC. Small RNA and transcriptional upregulation. Wiley Interdiscip Rev RNA 2011; 2:748-60; PMID:21823233; http://dx.doi.org/10.1002/wrna.90
58. Yin H, Lin H. An epigenetic activation role of Piwi and a Piwi-associated piRNA in Drosophila melanogaster. Nature 2007; 450:304-8; PMID:17952056; http://dx.doi.org/10.1038/nature06263
59. Nègre N, Brown CD, Ma L, Bristow CA, Miller SW, Wagner U, Kheradpour P, Eaton ML, Loriaux P, Sealfon R, et al. A cis-regulatory map of the Drosophila genome. Nature 2011; 471:527-31; http://dx.doi.org/10.1038/nature09990
60. Csink AK, Henikoff S. Something from nothing: the evolution and utility of satellite repeats. Trends Genet 1998; 14:200-4; http://dx.doi.org/10.1016/S0168-9525(98)01444-9
61. Ferree PM, Barbash DA. Species-specific heterochromatin prevents mitotic chromosome segregation to cause hybrid lethality in Drosophila. PLoS Biol 2009; 7:e1000234; PMID:19859525; http://dx.doi.org/10.1371/journal.pbio.1000234
62. Käs E, Laemmli UK. In vivo topoisomerase II cleavage of the Drosophila histone and satellite III repeats: DNA sequence and structural characteristics. EMBO J 1992; 11:705-16
63. Cugusi S, Ramos E, Ling H, Yokoyama R, Luk KM, Lucchesi JC. Topoisomerase II plays a role in dosage compensation inDrosophila. Transcription 2014; 4:238-50; http://dx.doi.org/10.4161/trns.26185
64. Furuhashi H, Nakajima M, Hirose S. DNA supercoiling factor contributes to dosage compensation in Drosophila. Development 2006; 133:4475-83; PMID:17035293; http://dx.doi.org/10.1242/dev.02620
65. Mendjan S, Taipale M, Kind J, Holz H, Gebhardt P, Schelder M, Vermeulen M, Buscaino A, Duncan K, Mueller J, et al. Nuclear pore components are involved in the transcriptional regulation of dosage compensation in Drosophila. Mol Cell 2006; 21:811-23; PMID:16543150; http://dx.doi.org/10.1016/j.molcel.2006.02.007
66. Vaquerizas JM, Suyama R, Kind J, Miura K, Luscombe NM, Akhtar A. Nuclear pore proteins nup153 and megator define transcriptionally active regions in the Drosophila genome. PLoS Genet 2010; 6:e1000846; PMID:20174442; http://dx.doi.org/10.1371/journal.pgen.1000846
67. Grimaud C, Becker PB. The dosage compensation complex shapes the conformation of the X chromosome in Drosophila. Genes Dev 2009; 23:2490-5; PMID:19884256; http://dx.doi.org/10.1101/gad.539509
68. de Wit E, de Laat W. A decade of 3C technologies: insights into nuclear organization. Genes Dev 2012; 26:11-24; PMID:22215806; http://dx.doi.org/10.1101/gad.179804.111

69. Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al. A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. Cell 2014; 159:1665-80; PMID:25497547; http://dx.doi.org/10.1016/j.cell.2014.11.021
70. Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, Hoichman M, Parrinello H, Tanay A, Cavalli G. Three-dimensional folding and functional organization principles of the Drosophila genome. Cell 2012; 148:458-72; PMID:22265598; http://dx.doi.org/10.1016/j.cell.2012.01.010
71. O'Hare K, Chadwick B, Constantinou A, Davis A, Mitchelson A, Tudor M. A 5.9-kb tandem repeat at the euchromatin-heterochromatin boundary of the X chromosome of Drosophila melanogaster. Mol Genet Genomics 2002; 267:647-55; http://dx.doi.org/10.1007/s00438-002-0698-x
72. Horakova AH, Moseley SC, McLaughlin CR, Tremblay DC, Chadwick BP. The macrosatellite DXZ4 mediates CTCF-dependent long-range intrachromosomal interactions on the human inactive X chromosome. Human Mol Genet 2012; 21:4367-77; http://dx.doi.org/10.1093/hmg/dds270
73. Ottaviani A, Rival-Gervier S, Boussouar A, Foerster AM, Rondier D, Sacconi S, Desnuelle C, Gilson E, Magdinier F. The D4Z4 macrosatellite repeat acts as a CTCF and A-type lamins-dependent insulator in Facio-Scapulo-Humeral dystrophy. PLoS Genet 2009; 5:e1000394; PMID:19247430; http://dx.doi.org/10.1371/journal.pgen.1000394
74. Rival-Gervier S, Lo MY, Khattak S, Pasceri P, Lorincz MC, Ellis J. Kinetics and epigenetics of retroviral silencing in mouse embryonic stem cells defined by deletion of the D4Z4 element. Mol Ther 2013; 21:1536-50; http://dx.doi.org/10.1038/mt.2013.131
75. Lei EP, Corces VG. A long-distance relationship between RNAi and Polycomb. Cell 2006; 124:886-8; PMID:16530034; http://dx.doi.org/10.1016/j.cell.2006.02.026
76. Kuhn E, Viering M, Rhodes K, Geyer P. A test of inuslator interactions in Drosophila. EMBO J 2003; 22:2463-71; http://dx.doi.org/10.1093/emboj/cdg241
77. Henikoff S, Ahmed K, Malik H. The centromere paradox: stable inheritance with rapidly evolving DNA. Science 2001; 293:1098-102; PMID:11498581; http://dx.doi.org/10.1126/science.1062939
78. Abad JP, Agudo M, Molina I, Losada A, Ripoll P, Villasante A. Pericentromeric regions containing 1.688 satellite DNA sequences show anti-kinetochore antibody staining in prometaphase chromosomes of Drosophila melanogaster. Mol General Genet MGG 2000; 264:371-7; PMID:11129040; http://dx.doi.org/10.1007/s004380000331
79. Larracuente AM. The organization and evolution of the Responder satellite in species of the Drosophila melanogaster group: dynamic evolution of a target of meiotic drive. BMC Evol Biol 2014; 14:233; http://dx.doi.org/10.1186/s12862-014-0233-9