

Chapter 1

Drosophila Satellite Repeats at the Intersection of Chromatin, Gene Regulation and Evolution



Maggie P. Lauria Sneideman and Victoria H. Meller

Abstract Satellite repeats make up a large fraction of the genomes of many higher eukaryotes. Until recently these sequences were viewed as molecular parasites with few functions. *Drosophila melanogaster* and related species have a wealth of diverse satellite repeats. Comparative studies of Drosophilids have been instrumental in understanding how these rapidly evolving sequences change and move. Remarkably, satellite repeats have been found to modulate gene expression and mediate genetic conflicts between chromosomes and between closely related fly species. This suggests that satellites play a key role in speciation. We have taken advantage of the depth of research on satellite repeats in flies to review the known functions of these sequences and consider their central role in evolution and gene expression.

Keywords Satellite DNA · Heterochromatin · Dosage compensation · Meiotic drive

1.1 Introduction

Repetitive DNA makes up a large portion of the genomes of higher eukaryotes. Satellite DNA, composed of tandem repeats that assemble into constitutive heterochromatin, was first described when mouse DNA was subjected to density gradient centrifugation and “satellite bands” of different densities formed above or below the bulk of the genome (Kit 1961; reviewed in Garrido-Ramos 2017). Fifty years ago the pioneering technique of in situ hybridization to mitotic chromosomes demonstrated that mouse satellite DNA was strikingly localized around the centromere (Pardue and Gall 1970). Although satellites propagate and may be mobile, expansion and movement are passive, relying on processes such as replication slippage, unequal crossing-over, or gene conversion to expand and move. This distinguishes satellites from transposable elements that typically insert as monomers and encode genes

M. P. Lauria Sneideman · V. H. Meller (✉)
Department of Biological Sciences, Wayne State University, Detroit, MI, USA
e-mail: Victoria.Meller@wayne.edu

necessary for mobilization. However, this dichotomy is not clean. Some satellite repeats may be derived from transposable elements (Dias et al. 2015; Meštrović et al. 2015). Both transposable elements and satellite repeats are enriched in heterochromatic regions, are subject to silencing by heterochromatin formation, and are often grouped with transposable elements for the purpose of analysis and discussion.

In spite of their abundance, satellite repeats are typically thought of as having few cellular functions besides contributing to the formation of heterochromatin, centromeres, and telomeres. But satellite DNA and RNA participate in a number of diverse processes, including gene regulation, stress response, and nuclear organization in *Drosophila melanogaster* and many other organisms. The mutability of satellites makes them prominent actors in the evolution of genomes. In accord with this, satellite repeats are a potent and adaptable weapon in genomic conflicts between species, and between chromosomes within a species. This review will focus on the functions of satellite repeats in *Drosophila* with particular attention to the properties that make satellites a versatile and powerful force in nuclear organization, gene regulation and evolution.

1.2 Seeing the Dark Matter of the Genome

Much of eukaryotic genomes are comprised of vast, uncharted blocks of heterochromatin surrounding centromeres and telomeres. These regions, made up of satellite repeats and transposable elements, resist cloning and have posed an insurmountable barrier to traditional methods of genome sequencing and assembly. But advances in long-read sequencing of unamplified DNA have allowed the most challenging regions of genomes to be assembled. Nanopore sequencing of high molecular weight DNA was used to complete human centromeres (Miga et al. 2020). PacBio sequencing has similarly enabled *Drosophila* centromeres to be assembled (Chang et al. 2019). These methods avoid bias in library preparation but have high error rates, making assembly of repetitive regions challenging. The performance of correction and assembly methods must consequently be validated before being used to reconstruct repetitive regions (Khost et al. 2017). At present, sequencing and assembly of major repetitive regions remains labor intensive and technically challenging. In contrast, the diversity and abundance of different types satellite repeats in the genome can be determined by sequencing of unamplified genomic DNA (Lower et al. 2018). This approach revealed differences in satellite composition between strains of *Drosophila melanogaster*, supporting the idea that satellites are a rapidly evolving portion of the genome (Wei et al. 2014). Interestingly, the satellite composition of different chromosomes is often distinct. This is observed in humans, where the variants of the α -satellite arrays that make up centromeres are chromosome specific (Rudd et al. 2006). It is also the rule in flies, where distinctive combinations of satellite repeats make up the pericentric heterochromatin of different chromosomes (Lohe et al. 1993; Blattes et al. 2006; Jagannathan et al. 2017; Chang et al. 2019).

Table 1.1 Major *D. melanogaster* satellite repeats

Sequence	%AT	Notable features	Citation
AATAT	100	Binds D1	1,8,12
AATAAAC	86		1,8
AATAG	80		1,8
AATAC	80		1,8
AATAACATAG	80	Prodsat, binds Prod	1,8,9
AATAGAC	71		1,8
AAGAC	60		1,8
AAGAG	60		1,8
TCAT	75		8
AAAAC	80		8
AACAC	60	Binds Lhr	8,11
AACAAAC	71		8
<i>Hsrω</i>	68	Stress induced, sequesters RNA processing factors	13
AAGAGAG	57		1,8
CCCGTACTCGGT	33	Dodeca, 11 and 12 bp variants, binds DP1	2,8,10
359 bp	69	X heterochromatin, binds D1 and topoisomerase 2, produces siRNA and lncRNA	1,3,4,5,8
1.688 ^x	65–72	359 bp variant in X euchromatin, guides dosage compensation	14
Rsp	71	Expansion on 2R, target of <i>Segregation distorter</i>	6,7
260 bp	71	2L heterochromatin	6,8

(1) Lohe et al. (1993), (2) Abad et al. (1992), (3) Dibartolomeis et al. (1992), (4) Waring and Pollack (1987), (5) Kuhn et al. (2012), (6) Khost et al. (2017), (7) Wu et al. (1988), (8) Jagannathan et al. (2017), (9) Török et al. (2000), (10) Huertas et al. (2017), (11) Satyaki et al. (2014), (12) Jagannathan et al. (2019), (13) Jolly and Lakhota (2006), (14) Menon et al. (2014)

1.3 Biophysical Properties of Satellites

Many satellites have interesting biophysical properties that are often commented on. How these contribute to function remains unclear in most instances. With the exception of *Dodeca*, *D. melanogaster* satellites are notably AT rich (Table 1.1). Indeed, AT richness is common in satellite DNA and may contribute to a curving of the duplex that enhances nucleosome stability (Fitzgerald et al. 1994; reviewed in Palomeque and Lorite 2008). Also suggestive is the observation that monomers of longer and more complex satellites often approximate the length of mono-, di-, or tri-nucleosomes, suggesting the potential for nucleosome phasing (Henikoff et al. 2001). For example, α -satellite repeats of human centromeres (171 bp) and the 359 bp satellite family of *D. melanogaster* suggest mono- and di-nucleosomes, respectively (Table 1.1). Nucleosomes are phased over the centromeric satellites of multiple species (reviewed in Heslop-Harrison and Schwarzacher 2013). The human centromere protein CENP-B enforces phasing by binding a 17 bp sequence in α -satellite repeats (Ando et al. 2002). The *Responder* (*Rsp*) repeats of

D. melanogaster, composed of two similar 120 bp units, also enforce phasing as demonstrated by an extended nucleosome periodicity of 240 bp (Doshi et al. 1991). The *Rsp* locus is known for its role as the target of meiotic drive, but the potential role of nucleosome phasing in this process is unknown. Taken together, these observations suggest that satellites display intrinsic features that are expected to contribute to nucleosome stability and influence the biophysical properties of chromatin.

1.4 How Do Satellites Expand, Move and Change?

D. melanogaster is rich in satellites, having approximately twice the diversity as humans (Shatskikh et al. 2020). The most abundant of these are 12 bp or less. As satellites generally lack coding potential their movement is nonautonomous. In spite of this limitation, they have been extraordinarily successful. Expansion and mobilization of satellite DNA reflects the propensities of replication and repair systems. For example, short tandem repeats are intrinsically unstable as they expand and contract by replication slippage (Fig. 1.1a) (Tautz et al. 1986; Bzymek and Lovett 2001; reviewed in Richards and Sutherland 1994; Levinson and Gutman 1987). Satellites also expand and contract by unequal crossing over during replication or repair (Fig. 1.1b). As longer, more complex monomers pose less of a challenge to the replication machinery, unequal crossing over is presumed to be a major factor variation of long repeats (Cabot et al. 1993; Southern 1975). A relevant question is why the expansion of noncoding sequence is tolerated. Some organisms have a considerably lower accumulation of satellite DNA, suggesting differences in susceptibility to slippage and unequal crossing over or tolerance of repetitive sequence. A comparison of related organisms with dramatic differences in genome size supports the idea that tolerance of additional genetic material is species specific (Petrov et al. 2000; Hartl 2000).

The movement of satellites may also occur by the formation of extrachromosomal loops that occur by recombination within an array. rDNA and noncoding tandem repeats are recovered as extrachromosomal loops in *Drosophila* and mammalian cells (Kiyama et al. 1986, 1987; Pont et al. 1987; Cohen et al. 2003, 2006; reviewed in Cohen and Segal 2009). This suggests a simple mechanism for movement to new sites. Extrachromosomal loops could undergo recombination with similar sequences or insert at random (Fig. 1.1c). The risk of extrachromosomal loops to genome integrity is moderated by the assembly of satellite repeats into heterochromatin. In accord with this idea, the loss of heterochromatin factors elevates the level of extrachromosomal loops and increases genomic damage (Larson et al. 2012; Peng and Karpen 2007). The erosion of heterochromatic silencing that is observed in aging and cancer is presumed to lead to the increase in extrachromosomal loops and

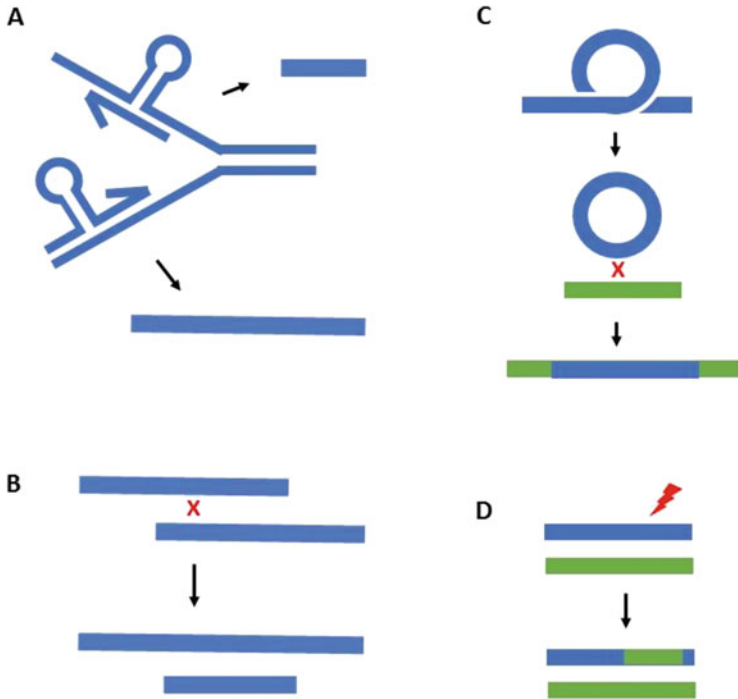


Fig. 1.1 The repetitive structure of satellite repeats facilitates movement and change. (a) Replication stalling at repeats allows mispairing and template slippage. This produces contraction (top) or expansion (bottom) of tandem repeats. (b) Unequal crossing over leads to the expansion and contraction of tandem arrays. Cycles of unequal crossing over homogenize repeats at the center of an array. (c) Extrachromosomal loops generated by recombination within a tandem array can insert at a new site. (d) Gene conversion occurs when a related sequence serves as a template for recombination or repair

contribute to genome instability in these cells (Sinclair and Guarente 1997; Larson et al. 2012; Turner et al. 2017; deCarvalho et al. 2018; Kim et al. 2020).

Tandem arrays are also subject to gene conversion by recombination within a cluster or with similar sequences from elsewhere in the genome (Fig. 1.1d). This general process is of interest for its role in the evolutionary divergence of duplicated genes (Osada and Innan 2008). The extreme abundance of satellite repeats that could be used as templates favors this process but raises the potential for large, damaging genome rearrangements. The idea that protection of repetitive DNA from inappropriate recombination is one of the functions of heterochromatin is supported by the behavior of repair foci in heterochromatic regions (Caridi et al. 2018). These foci move out of the nuclear territory occupied by heterochromatin before completion of the repair, supporting the idea that recombination and repair of repetitive DNA are potentially dangerous and under tight control.

1.5 Evolution of Satellite Repeats Is Rapid and Driven

Closely related species often display striking variations in satellite repeat composition and abundance (Bosco et al. 2007; Jagannathan et al. 2017; Lohe and Brutlag 1987). A comparative study of the satellite composition of four closely related species, *D. melanogaster*, *D. sechellia*, *D. simulans*, and *D. mauritiana* used hybridization to mitotic chromosomes to compare satellite composition and localization (Jagannathan et al. 2017). Some classes of satellites undergo complete replacement in closely related species. For example, Prodsat (AATAACATAG, Table 1.1) makes up 2% of the *D. melanogaster* genome but is not detected in the other three species (Török et al. 2000; Jagannathan et al. 2017). One caveat of this approach is that sites with low copy numbers of repeats are below the detection limit on mitotic chromosomes. For example, several megabases of 359 bp satellites in pericentromeric heterochromatin on the *D. melanogaster* X are detected by this method, but hundreds of closely related satellites dispersed throughout X euchromatin are not.

Dispersed satellites in euchromatin have also been subject to rapid, widespread changes in sequence, position, and abundance (Sproul et al. 2020). Although striking, this wholesale replacement is the natural outcome of two mutagenic processes with vastly different speeds. Point mutations diversify sequence, and the accumulation of mutations will eventually destroy the identity between sequences derived from the same progenitor. In contrast, gene conversion occurs orders of magnitude much more rapidly and acts to homogenize repeats within an array, and between arrays at different sites in the genome (Ohta and Dover 1984). The outcome of these competing mutational processes is the replacement and homogenization of satellites throughout the genome, termed molecular drive (Dover 1982). This is particularly dramatic when comparing closely related species, such as the Drosophilids (Jagannathan et al. 2017; Sproul et al. 2020; de Lima et al. 2020; Larracuente 2014).

1.6 Satellites, Silencing, and Organization of Chromatin

One of the most prominent features of satellite repeats is their role in heterochromatin formation. Large arrays of tandem repeats trigger silencing through heterochromatin formation that is largely sequence independent (Henikoff 1998). This has bedeviled mouse genetic studies because random transgene insertions produce tandem arrays subject to silencing. Using Cre/LoxP to excise extra copies from a mouse transgene array, Garrick et al. (1998) demonstrated that chromatin compaction and transgene silencing was not an intrinsic feature of the insertion site or transgene sequence, but was instead induced by multicopy arrays. Silencing of tandem transgenes is also observed in flies and plants, indicative of a common strategy for inactivating repetitive DNA (Dorer and Henikoff 1994). As most repetitive sequences are potential threats to genome integrity, the recognition and silencing of repeats represent a triumph of genome defense.

RNA derived from transposable elements and satellites direct the chromatin modifications that initiate heterochromatin formation in fission yeast and this serves as a useful model for the process (reviewed by Grewal and Elgin 2007). Transcription through repeats generates RNAs that are processed into siRNAs and loaded onto Argonaute effector complexes (Höck and Meister 2008). Nascent RNA from cognate regions of the genome is bound by these complexes, which recruit a histone methyltransferase that places the H3K9me mark. The heterochromatin protein Swi6, and a number of small RNA processing factors, are recruited by H3K9me to ensure maintenance of silencing (Zhang et al. 2008). While *Drosophila* heterochromatin is heterogeneous, evidence suggests that small RNA pathways also contribute to chromatin regulation in flies (Swenson et al. 2016; Cernilogar et al. 2011). Mislocalization of heterochromatin proteins and break down of silencing have been observed when Argonaute effectors or the genes necessary to produce small RNAs are inactivated (Fagegaltier et al. 2009). A genetically distinct silencing system in the germ line controls transposable elements by message destruction and transcriptional silencing (Khurana et al. 2010). This is directed by Piwi RNAs (piRNAs), generated from transposon sequences archived in piRNA clusters and expressed in the germ line (Brennecke et al. 2007). The resulting piRNAs enable Piwi, a germ line-specific Argonaute protein, to identify and bind nascent transcripts from mobile elements. Piwi recruits an H3K9 methyltransferase through an adapter protein to establish silencing (Sienski et al. 2015). Components of the Piwi system are also involved in chromatin compaction and silencing at later developmental stages. Maternal depletion of Piwi impairs heterochromatic silencing in the adult, a long-lasting effect that is observed by reduction of Position Effect Variegation (PEV) (Gu and Elgin 2013). PEV occurs when transgenes in repressive environments are silenced in some cells (Elgin and Reuter 2013). The majority of piRNAs have the identity to transposons, consistent with their vital role in the repression of mobile elements (Brennecke et al. 2007). But satellite piRNA are also present and may direct heterochromatin compaction of some repeats in the early embryo. Maternally deposited cues, possibly small RNA, direct the formation of zygotic heterochromatin over a cluster of 359 bp satellites on the X chromosome (Ferree and Barbash 2009; Yuan and O'Farrell 2016). The 359 bp satellites are notable for their role in hybrid incompatibility between closely related species, discussed in a following section.

Heterochromatin itself displays remarkable biophysical properties. Visualization of *D. melanogaster* heterochromatin reveals a subnuclear compartment that is distinct from euchromatin and which may consolidate the major heterochromatic regions of all chromosomes (see Caridi et al. 2018). The discovery that fly and human HP1 phase separate in vitro, and that heterochromatin itself displays the properties of phase separation in cells, suggested a biophysical explanation for how segregation is achieved (Strom et al. 2017; Larson et al. 2017). Phase separation of subcellular bodies occurs by the self-association of disordered proteins (reviewed in Hall et al. 2019). Separation is favored by multivalent interactions, protein crowding, and assembly with a polymer, such as RNA or chromatin. HP1 has disordered domains, interacts with a large number of proteins and also binds RNA (Alekseyenko et al. 2014; Muchardt et al. 2002; Roach et al. 2020). A functional

role for RNA in HP1 localization is suggested by the finding that HP1a is released from mouse nuclei by RNase and the association of fly HP1a with chromatin is also RNA dependent (Maison et al. 2002; Piacentini et al. 2003).

Many chromatin proteins in addition to HP1 have RNA-binding domains or interact with RNA-binding proteins, in a manner that suggests a structural role for RNA in chromatin organization. One of these, Decondensation factor 31 (Df31), a small, hydrophobic, and highly disordered RNA binding protein, also boasts a large protein–protein interaction network. The general distribution of Df31 in the nucleus suggests a role in maintaining chromosome territories (Rohrbaugh et al. 2013). In cultured *Drosophila* cells association of Df31 with RNA is necessary for accessible chromatin (Schubert et al. 2012). In vitro assays found that Df31 association with chromatin was RNA dependent and RNase treatment collapsed chromatin into a nuclease-resistant state. While Df31 shows hallmarks of a protein involved in phase separation, it is enriched in euchromatic regions.

Scaffold Attachment Factor A (SAF-A, HNRNPU in humans) and SAF-B have DNA binding domains that recognize AT-rich matrix or scaffold attachment sites (Fackelmayer et al. 1994; Göhring and Fackelmayer 1997; Nozawa et al. 2017; Fan et al. 2018). These similar proteins also have RNA binding domains and large disordered regions. Loss of these proteins disrupts chromatin structure and DNA accessibility, as does RNase digestion (Nickerson et al. 1989; Nozawa et al. 2017; Fan et al. 2018). Mouse SAF-B binds a variety of long noncoding RNAs, but transcripts from pericentric satellite repeats are its predominant partners (Huo et al. 2020). Depletion of mouse SAF-B allowed heterochromatin bodies in the nucleus to expand and make interchromosomal contacts. Imaging reveals that SAF-B coats the exterior of H3K9me3-rich heterochromatin bodies, suggesting a SAF-B shell that prevents inappropriate mingling of phase-separated heterochromatin domains from different chromosomes (Huo et al. 2020). *Drosophila* SAF-B binds chromatin and is also visualized as an extrachromosomal network (Alfonso-Parra and Maggert 2010). The Association of fly SAF-B with chromatin responds to transcription and is differentially affected by mutation of its DNA binding domain and RNase treatment, but whether or not fly SAF-B interacts with specific RNAs is unknown.

Responses to heat shock and stress suggest that satellite RNA is situated in an interconnected web of RNA-binding proteins that organize chromatin and coordinate mRNA processing. When mammalian cells are subjected to stress, transcription of Sat III RNA is dramatically upregulated (Rizzi et al. 2004). SAF-B, and many RNA-binding factors involved in message processing, are recruited to nuclear stress bodies that form at sites of Sat III transcription (Valgardsdottir et al. 2008). Knock-down of Sat III RNA partially reversed the transcriptional repression induced by heat shock, suggesting a mechanism for rapidly restructuring chromatin and RNA processing pathways during stress (Goenka et al. 2016). In flies the *Heat shock RNA omega* (*Hsr ω*) RNA serves a similar function. This noncoding transcript orchestrates stress response by sequestering splicing and RNA processing factors (reviewed by Jolly and Lakhotia 2006). *D. melanogaster Hsr ω* includes 20 kb of AT-rich, 280 bp tandem repeats, thus conforming to the pattern of AT-rich satellites with a repeat length corresponding to multiples of nucleosome length.

1.7 Tandem Repeats in Euchromatin Modulate Nearby Genes

The role of satellite repeats in nucleating heterochromatin formation is well known, but tandem repeats of all types, including satellites, play interesting and surprising roles in gene regulation in euchromatin. A portion of satellite DNA is distributed throughout the euchromatic genome in tandem arrays. Changes in the number of repeats have created a wealth of genetic variation that has been exploited in forensic analysis, population genetics, and conservation. Although often considered neutral, microsatellites are highly represented in the promoters of human genes (Sawaya et al. 2013; Tomilin 2008). Dinucleotide repeats are enriched in fly enhancers, where they contribute to normal expression levels (Yanez-Cuna et al. 2014). These authors concluded that the association of short tandem repeats with regulatory regions is broadly conserved. Roughly 25% of the promoters in baker's yeast, *Saccharomyces cerevisiae*, also contain tandem repeats (Vinces et al. 2009). These increase gene expression as the length of the repeats expanded. Tandem repeats also mediate repression. In the beetle *Tribolium castaneum* a major satellite DNA family near euchromatic genes maintains repression after heat stress (Felicciello et al. 2015). The mutability of short tandem repeats suggests a potential source of phenotypic variation. In accord with this idea, variation in repetitive DNA has been linked to expression differences in plants and insects (Ranathunge et al. 2018; Brajković et al. 2012). Repeat length variations in developmental genes, coupled with selection by breeders, are responsible for rapid phenotypic evolution in dogs (Fondon and Garner 2004). Social behavior in voles is influenced by satellite polymorphisms in a vasopressin receptor and length variants of repeats in the *period* (*per*) gene of *D. melanogaster* determine the male courtship song rhythm (Yu et al. 1987; Hammock and Young 2005). In addition to providing a source of genetic diversity, satellite repeats have been recruited to wage genomic conflicts and enable sex chromosome dosage compensation, described in the following sections. Their usefulness in these contexts owes to the properties described above: mobility, rapid evolution, and multifaceted roles in the structure and regulation of chromatin.

1.8 Chromosome Identification During Dosage Compensation

Organisms with highly differentiated sex chromosomes, such as humans and *Drosophila*, must address the problem of sex chromosome gene dosage. Males are functionally hemizygous for X-linked genes. Flies meet this challenge by increasing expression from virtually every gene on the single male X chromosome to match that of the two female X chromosomes. The Male-Specific Lethal (MSL) complex, composed of five proteins and one of two redundant RNAs, is essential for this process (reviewed in Kuroda et al. 2016). The MSL complex is selectively recruited

to actively expressed X-linked genes (Alekseyenko et al. 2006; Bell et al. 2008; Sural et al. 2008). One of the MSL proteins, Males absent on the first (Mof), is a histone acetyltransferase that deposits the H4K16ac mark within the gene body (Kind et al. 2008; Copur et al. 2018). Histone acetylation increases the likelihood that initiated transcripts will be completed, raising the level of transcripts approximately twofold (Larschan et al. 2011). A long noncoding RNA, *roX1* or *roX2*, must be part of the complex for proper X localization (Meller and Rattner 2002). Severe *roX1 roX2* mutants are male lethal, the expression of X-linked genes is reduced and MSL proteins localize to ectopic autosomal sites (Deng and Meller 2006). How the MSL complex identifies the X chromosome with the required selectivity is still unknown. Studies in a number of laboratories characterized Chromatin Entry Sites (CES) on the X chromosome that bind an adapter protein and recruit the MSL complex directly (Alekseyenko et al. 2008; Straub et al. 2008; Soruco et al. 2013). However, the adapter protein binds related sites on all chromosome arms but only recruits the MSL complex in the context of X-linked sites. This suggests the presence of additional X identity elements.

The striking enrichment of a clade of 359 bp repeats, termed the 1.688^X repeats (Table 1.1) in X euchromatin pointed to a potential role in an X chromosome-specific process such as dosage compensation (Hsieh and Brutlag 1979; Waring and Pollack 1987; Dibartolomeis et al. 1992). The 1.688^X repeats are enriched near genes, including promoters and introns, leading to the suggestion that they could modulate expression (Kuhn et al. 2012). Autosomal insertions of short clusters of these repeats induced recruitment of the MSL complex and partial compensation of genes as much as 140 kb away (Joshi and Meller 2017; Deshpande and Meller 2018). A clue to how these repeats function came from the discovery that mutations in the siRNA pathway enhanced the lethality of males with partial loss of function *roX1* and *roX2* chromosomes (Menon and Meller 2012). Furthermore, ectopic expression of siRNA from one 1.688^X repeat partially restored MSL localization and rescued *roX1 roX2* males (Menon et al. 2014). Taken together, these studies reveal that the 1.688^X satellite repeats are X identity elements and suggest that the siRNA pathway mediates their function. Interestingly, other *Drosophilid* X chromosomes are highly enriched for chromosome-specific repeats, although the sequence of these repeats is not highly conserved (Gallach 2014). Particularly striking is the rapid acquisition of repeats by neo-X chromosomes that arise by the fusion of an autosome to a sex chromosome. These fusions also produce a neo-Y, fated to degenerate as it passes exclusively through males without recombination (reviewed by Wei and Barbash 2015). Degeneration of the neo-Y necessitates compensation of genes on the neo-X. The relative mobility of satellite repeats makes them well suited for marking a young X chromosome to enable it to capture the dosage compensation machinery.

The manner in which the 1.688^X satellites identify the X chromosome remains unknown, but there are clues that the mechanism is very different than that of the CES. The 1.688^X satellites on the X chromosome are not generally enriched for the MSL proteins, suggesting that they do not recruit directly but mark the X chromosome in some fashion (Deshpande and Meller 2018). Ectopic expression of 1.688^X siRNA increased the repressive H3K9me2 mark on autosomal 1.688^X satellite

insertions (Deshpande and Meller 2018). Contrary to conventional expectations for a repressive mark, this increased the expression of nearby genes in males. The finding that HP1 is modestly enriched on the male X chromosome, and that mutations in several heterochromatin factors selectively disrupt the structure of the polytenized male X, support the idea that repressive marks are in some way linked to fly dosage compensation (Spierer et al. 2005, 2008; De Wit et al. 2005). How satellite repeats and repressive marks might accomplish this is speculative, but one possibility is through influencing chromatin organization in the nucleus. Nuclear organization is a factor in X chromosome compensation in other organisms. For example, the single male X chromosome of *C. elegans* is located at the periphery of the nucleus, but the two female X chromosomes are centrally located (Sharma et al. 2014). Interaction with nuclear pore proteins may elevate the expression of X-linked genes in this species. A role for nuclear pore proteins in MSL loading and activation has also been proposed in flies (Mendjan et al. 2006). More generally, the ability of X-linked genes to acquire dosage compensation during development is attributed to the three-dimensional organization of X chromosomes in the nucleus of mammals and flies (Engreitz et al. 2013; Schauer et al. 2017; Ramírez et al. 2015). A role of the location or organization of the X chromosome is one way that 1.688^X satellites might promote X recognition.

1.9 Satellites and Centromeres

The most widely appreciated function of satellite DNA is at centromeres. Human centromeres contain α -satellite arrays harboring a motif that interacts with the centromeric H3 variant CENP-A, suggesting determination by sequence (Masumoto et al. 1989; reviewed by McNulty et al. 2017; Willard 1985; Schueler et al. 2001). However, human centromeres occupy only part of the array and satellites are absent from some neo-centromeres, challenging the idea that sequence is the primary centromere determinant. Fly centromeres also form within extensive arrays of satellite repeats, but the centromere itself assembles at “islands” of transposons embedded in this sea of satellites (Chang et al. 2019). Fly centromeres are defined by the incorporation of an H3 variant called Cid. The importance of epigenetic information in specifying centromeres in flies is demonstrated by the fact that the transposons at the fly centromere are by no means limited to the centromere, appearing in both heterochromatic and euchromatic contexts throughout the genome (Chang et al. 2019). It is also reflected in the persistence of a functional centromere following transient anchoring of the centromere-specific chaperone CAL-1, which is capable of loading Cid at ectopic sites (Chen et al. 2014). These findings indicate that both fly and human centromeres are specified by a combination of DNA sequence, genomic context, and epigenetic marking. Centromeres are surrounded by heterochromatin that contributes to their function. A large deletion of heterochromatin flanking a fly centromere produced mitotic instability and premature sister chromatid separation (Wines and Henikoff 1992). This is consistent with the enrichment of cohesin in heterochromatin and suggests that the mitotic machinery is tuned to a

certain arrangement of heterochromatin surrounding the centromere (Bernard and Allshire 2002).

RNA from satellites has also been found to localize to centromeres. Transcripts from a large block of pericentric 359 bp satellites on the *D. melanogaster* X chromosome bind *in cis* to the centromeric region (Bobkov et al. 2018). RNA from the mammalian α -satellite also binds to centromeric proteins and localizes *in cis* at centromeres (Wong et al. 2007; reviewed in Ideue and Tani 2020; McNulty et al. 2017). This RNA is also necessary for characteristic localization of centromeric proteins CENPC1 and INCENP, and so may function to recruit or stabilize components of the centromere.

1.10 Satellites Are the Ammunition of Genomic Conflicts

Chromosomes are fundamental units of inheritance and take an active role in biasing their own transmission to the next generation. Meiotic drive, when a genetic element manipulates reproduction to favor its own transmission and overthrow Mendel's rules, is the outcome. A selfish chromosome able to accomplish this will increase in the population. Evolutionary theory posits that systems of meiotic drive emerge frequently and sweep through the population, driving enrichment of one chromosome and limiting genetic variation. In addition, unfavorable, genetically linked alleles are allowed to proliferate (Courret et al. 2019). This extracts a cost in fitness that enables suppressors of drive to emerge and restore Mendelian segregation. A history of recurring cycles of drive and suppression is revealed when wild-caught flies are outcrossed and suppressed drivers emerge (Hartl and Hartung 1975). These conflicts are often mediated through satellite repeats and the outcome shapes the genome.

Sex differences in meiosis ensure that a strategy for biasing chromosome transmission can only function in one sex. While all products of male meiosis have the potential to develop into sperm, only one of the four products of female meiosis will become the egg. To gain an advantage in female meiosis the critical point is the alignment of homologs on the spindle at the first division (Fig. 1.2a). A centromere that attaches to the egg pole will escape elimination in the polar body (Rosin and Mellone 2017; Kursel and Malik 2018). To take advantage of this requires asymmetry in the meiotic spindle and a centromere able to exploit the asymmetry. The extraordinary reproductive advantage that a stronger centromere holds is thought to fuel an evolutionary race that drives rapid changes in centromeric DNA and proteins (Malik 2009). Predictions of this model are fulfilled in mice where an expansion of satellite repeats has produced a large centromere with an advantage over a homolog with fewer satellites (Iwata-Otsubo et al. 2017). Interestingly, the kinetochores of the larger centromere detach more frequently from cortical spindle fibers, providing an opportunity to reattach to the egg pole (Akeru et al. 2019). Suppressors of centromere drive would benefit a population in which a chromosome had begun to cheat. The observation that the centromeric variants Cid and CEN-A are remarkably fast-evolving and divergent from other histones suggests their involvement (Black et al.

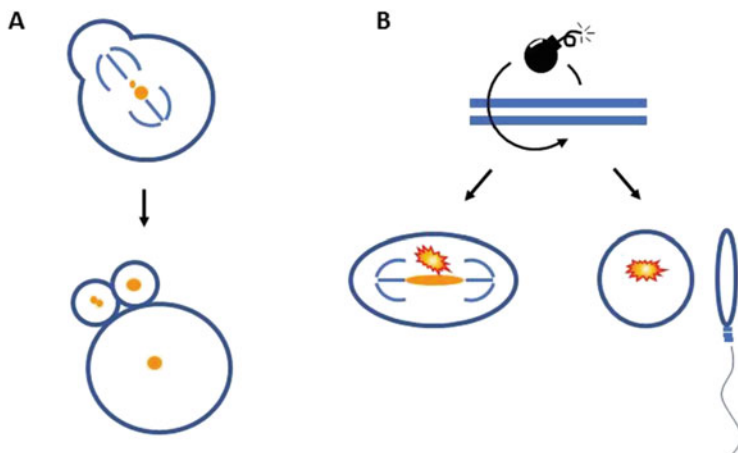


Fig. 1.2 Meiotic drive in female and male germlines. (a) Stronger centromeres (larger dot) gain an advantage in the female germline by avoiding the cortical spindle and becoming an egg nucleus more than 50% of the time. (b) Chromosomes that achieve drive in males do so by sabotaging their homolog. This may be direct, as implied in the cartoon, or indirect by the establishment of an environment that is toxic to cells inheriting the susceptible homolog. Left) The Paris sex ratio X chromosome produces a factor that blocks segregation of the Y at the second meiotic division. Failure to form Y-bearing sperm ensures predominantly female broods. Right) The *Sd* and Winters drivers sabotage the maturation of sperm carrying susceptible homologs. In both systems, arrest occurs before chromatin compaction and these malformed cells are eliminated (center). Sperm carrying the driver (right) develop normally

2004). Amino acid changes in Cid are concentrated in a region that interacts with H4 and an extended loop that contacts DNA (Vermaak et al. 2002). The rapid evolution of centromeres and the proteins that bind them seems at odds with the very conservative function of centromeres but is in accord with the idea that these structures are the site of an evolutionary battle (Malik 2009).

All products of male meiosis have the opportunity to become sperm. To gain an advantage in the male germ line a chromosome must exert a negative effect on its homolog (Fig. 1.2b). Several examples of this are well known, including *Segregation distorter/Responder* (*Sd/Rsp*) in *D. melanogaster*. *Rsp*, the target of drive, is an array of two very similar 120 bp repeats present in dozens to thousands of copies (Khost et al. 2017). Larger arrays confer increased sensitivity to *Sd* (Larracunte and Presgraves 2012; Moschetti et al. 1996). *Sd* is a truncated but enzymatically active duplication of RanGAP that mislocalizes to the interior of the nucleus (Kusano et al. 2001). When a male has *Sd* on one homolog and a sensitive *Rsp* allele (*Rsp^S*) on the other, maturation of sperm carrying *Rsp^S* is arrested and these cells are eliminated. The sperm carrying *Sd* develop normally and are responsible for most or all fertilizations. The *Sd* phenotype is enhanced by a number of modifiers, all genetically linked to *Sd* on the second chromosome (reviewed by Larracunte and Presgraves 2012). Of course, *Sd* chromosomes must themselves carry insensitive *Rsp* arrays in order to escape elimination. Although the precise molecular defect that

causes arrest is unclear, abnormal localization of mutant RanGAP is thought to disrupt the RanGTP/GDP gradient across the nuclear envelope and this may interfere with transport in and out of the nucleus (Kusano et al. 2003). In this environment, the expanded repeats on *Rsp*^S chromosome precipitate failure of sperm maturation. The process that is affected must be unique to sperm development as *Sd/Rsp*^S females produce normal offspring ratios. During sperm maturation chromatin is remodeled by the replacement of histones, a process requiring the import of protamine. This step is male-limited and appropriate to the stage of arrest, but it is unclear how expanded *Rsp*^S arrays and defects in protamine levels would induce arrest. Disruption of small RNA import leading to a defect in repackaging *Rsp* chromatin is also possible. Small RNAs from *Rsp* have been identified in the germ line and mutations in *aubergine* (*aub*), an argonaute family protein that participates in piRNA production, enhances distortion by *Sd* (Gell and Reenan 2013; Nagao et al. 2010). This finding suggests that an additional role of germ line small RNA systems is to defend against meiotic drive.

Rsp provides an excellent example of satellite turnover. Two families of repeats, the *Rsp* and *Rsp-like* family and the 359 bp family, which includes an extensive array of pericentromeric 359 bp repeats in X heterochromatin and the euchromatic 1.688^X satellites, were found to occupy overlapping sites in related species (Sproul et al. 2020). Both the *Rsp* and 359 families are AT-rich, but the 359 bp repeats are widespread, older, and more diversified in related species. Examination of satellite repeats in several *Drosophila* species revealed that 1.688^X and *Rsp-like* satellites occupy many of the same euchromatic sites (Sproul et al. 2020). Sites in which *Rsp-like* repeats have been inserted in an existing 1.688^X array, possibly in the process of replacing it, were identified in *D. simulans* and *D. mauritiana*. This suggests a model in which young *Rsp-like* repeats use homology with existing 1.688^X repeats to enter these sites, a process that may be facilitated by long-range interactions in the nucleus. Extrachromosomal circular DNAs are also a potential mechanism for movement. The correlation between the abundance of one of the repetitive elements and extrachromosomal DNA also suggests a role in *Rsp-like* invasion (Sproul et al. 2020).

D. simulans has at least three meiotic drive systems that bias sex chromosome inheritance and thus distort the sex ratio. All of these involve drivers on the X chromosome. In the Winters system, named for the location where the flies were collected, the X chromosome distorter prevents Y-bearing gametes from completing maturation. Failure occurs during condensation of the haploid nucleus, a timing that is similar to that observed in the *D. melanogaster Sd/Rsp* system (Tao et al. 2007b). The driver, *Distorter on the X chromosome* (*Dox*), is a partial duplication that produces an RNA with limited coding potential (Tao et al. 2007a). The mechanism of *Dox* action is unknown but suppressors of *Dox* on the second chromosome generate siRNAs that reduce levels of the *Dox* transcript (Lin et al. 2018). As the Y chromosome is primarily composed of satellite repeats and transposons, it is likely that the toxic effect of *Dox* depends on the unique sequence and chromatin composition of this chromosome. A second *D. simulans* sex ratio distortion system, Paris, induces anaphase bridges and failure of Y chromosome disjunction during the

second meiotic division (Fig. 1.2b, Cazemajor et al. 2000). One component of the X-linked driver was discovered to be a loss of function mutation in a rapidly evolving member of the *HP1* family, *HP1D2* (Helleu et al. 2016). Intriguingly, the HP1D2 protein is specifically enriched on the Y chromosome, suggesting that a defect in the organization or compaction of this chromosome prevents segregation. Sex ratio distortion leads to populations with unbalanced ratios of males and females and creates a strong selective advantage for an individual with a novel suppressor of drive. In accord with this, Y chromosomes that are resistant to the Paris or Winters driver have been discovered (Branco et al. 2013; Helleu et al. 2019). As the coding potential on the Y is limited, it is quite possible that these suppressors are changes in satellite or transposon content that make them insensitive to the X-linked driver.

1.11 Satellite Repeats Mediate Conflict Between Species

Hybrid incompatibilities enforce the reproductive isolation that defines species (Castillo and Barbash 2017). The rapid evolution of heterochromatin DNA and proteins is a potential source of incompatibilities that produce lethality or infertility upon hybridization of closely related Drosophilids (Presgraves 2010; Ferree and Barbash 2009; Gatti et al. 1976; Yunis and Yasmineh 1971; reviewed in Ferree and Prasad 2012). For example, when *D. melanogaster* males are mated to *D. simulans* females, male offspring emerge as sterile adults but females die as embryos. Early female lethality is attributable to the *D. melanogaster* X chromosome in *D. simulans* cytoplasm. Specifically, the large array of 359 bp repeats at the base of the *D. melanogaster* X chromosome fails to compact and X chromatids become entangled in anaphase bridges (Ferree and Barbash 2009). But when *D. melanogaster* males transmitted a *Zygotic hybrid rescue* (*Zhr*) chromosome that was deleted for the pericentric 359 bp repeats, female offspring survived (Sawamura et al. 1993). Small RNAs from the 359 bp satellite are present in oocytes from *D. melanogaster* females, and it is plausible that these direct heterochromatin formation over the 359 bp satellites in fertilized embryos (Ferree and Barbash 2009). The X chromosome of *D. simulans* lacks 359 bp repeats and the relevant class of siRNA is not present in *D. simulans* eggs. These ideas are supported by a study demonstrating that heterochromatin formation at the 359 bp satellites occurred with different timing than that of another large satellite array and required maternal factors missing from *D. simulans* ooplasm (Yuan and O'Farrell 2016).

The reciprocal mating, *D. melanogaster* females mated to *D. simulans* males, produced sterile female adults but no male offspring. The toxic interaction producing male lethality can be traced to heterochromatin proteins, *Lethal hybrid rescue* (*Lhr*, *D. simulans*) and *Hybrid male rescue* (*Hmr*, *D. melanogaster*) (Maheshwari and Barbash 2012). Higher expression of *Lhr* from the *D. simulans* chromosome is the basis of hybrid lethality. Loss of *D. simulans* *Lhr* rescues hybrid lethality, but loss of *D. melanogaster* *Lhr*, which is expressed at only half the rate as *D. simulans*, does not achieve rescue. *Lhr* encodes a rapidly evolving heterochromatin protein that

interacts with HP1 (Brideau et al. 2006; Brideau and Barbash 2011; Thomae et al. 2013). Loss of *Lhr* results in bloated polytene chromosomes in *D. simulans*, a phenotype associated with a loss of chromosome structure (Pal Bhadra et al. 2006). The *Hmr* gene is also rapidly evolving and encodes a DNA binding protein that localizes to heterochromatin in a complex with Lhr and HP1a (Satyaki et al. 2014; Alekseyenko et al. 2014). Hybrid males, which die as larvae, display poorly condensed chromosomes and anaphase bridges between sister chromatids, consistent with the idea that heterochromatin assembly and compaction is the primary defect (Blum et al. 2017).

The conflicts between genetic elements within a species that produce meiotic drive, and between species that lead to hybrid incompatibility, rely on an overlapping cast of characters. In accord with this, it has been suggested that meiotic drive contributes to the genetic divergence that produces hybrid incompatibility (McDermott and Noor 2010). This notion is supported by the discovery that a single gene, *Overdrive* (*Ovd*), appears responsible for both meiotic drive and hybrid incompatibility in *D. pseudoobscura* (Phadnis and Orr 2009). Males from a mating between subspecies are sterile when young but become weakly fertile and produce almost exclusively daughters when aged. Although the molecular mechanisms at play are currently unknown, the finding that one gene is involved in both phenomena supports the idea that meiotic drive and hybrid incompatibility are produced by similar genetic conflicts.

Summary

Satellite repeats appeared both troublesome and singularly unpromising at the dawn of the genomics era. The typical concentration of satellites in vast, unclonable blocks of heterochromatin was an additional deterrent to their study. But the ability of satellites to move, expand, and undergo relatively rapid genome-wide replacement enables them to shape genomes and respond to evolutionary pressures. Satellite DNA, and small RNA pathways capable of directing modifications to chromatin, are a powerful combination that can be adapted to novel roles. This can be appreciated by the dispersed, euchromatic 1.688^X satellites that recruit dosage compensation while very similar heterochromatic 359 bp satellites mediate hybrid incompatibility at a different life stage and in different sex. In spite of the stark differences in the roles of these satellites, it is likely that small RNA normally directs chromatin modifications to both, and that these modifications are essential for normal function. When heterochromatin is compromised satellites become unstable and devastating disruptions of nuclear organization result. This intrinsic risk can be appreciated by the destruction unleashed by 359 bp satellites in a hybrid environment. The remarkable ability of heterochromatin to assemble satellite DNA into a nondestructive form can be credited with enabling satellite repeats to expand to their current position of prominence in higher eukaryotes. All of the properties described above, including the mutability of satellites and their intrinsic danger, put repetitive sequences at the leading edge of evolution.

References

- Abad JP, Carmena M, Baars S, Saunders RD, Glover DM, Ludeña P, Sentis C, Tyler-Smith C, Villasante A (1992) Dodeca satellite: a conserved G+C-rich satellite from the centromeric heterochromatin of *Drosophila melanogaster*. PNAS 89:4663–4667. <https://doi.org/10.1073/pnas.89.10.4663>
- Akera T, Trimm E, Lampson MA (2019) Molecular strategies of meiotic cheating by selfish centromeres. Cell 178(5):1132–1144.e10. <https://doi.org/10.1016/j.cell.2019.07.001>
- Alekseyenko AA, Larschan E, Lai WR, Park PJ, Kuroda MI (2006) High-resolution ChIP-chip analysis reveals that the Drosophila MSL complex selectively identifies active genes on the male X chromosome. Genes Dev 20(7):848–857. <https://doi.org/10.1101/gad.1400206>
- Alekseyenko AA, Peng S, Larschan E, Gorchakov AA, Lee OK, Kharchenko P, McGrath SD, Wang CI, Mardis ER, Park PJ, Kuroda MI (2008) A sequence Motif within chromatin entry sites directs MSL establishment on the drosophila X chromosome. Cell 134(4):599–609. <https://doi.org/10.1016/j.cell.2008.06.033>
- Alekseyenko AA, Gorchakov AA, Zee BM, Fuchs SM, Kharchenko PV, Kuroda MI (2014) Heterochromatin-associated interactions of Drosophila HP1a with dADD1, HIPPI1, and repetitive RNAs. Genes Dev 28(13):1445–1460. <https://doi.org/10.1101/gad.241950.114>
- Alfonso-Parra C, Maggert KA (2010) Drosophila SAF-B links the nuclear matrix, chromosomes, and transcriptional activity. PLoS One 5(4). <https://doi.org/10.1371/journal.pone.0010248>
- Ando S, Yang H, Nozaki N, Okazaki T, Yoda K (2002) CENP-A, -B, and -C chromatin complex that contains the I-type α -satellite array constitutes the prekinetochore in HeLa cells. Mol Cell Biol 22(7):2229–2241. <https://doi.org/10.1128/mcb.22.7.2229-2241.2002>
- Bell O, Conrad T, Kind J, Wirbelauer C, Akhtar A, Schübeler D (2008) 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 28(10):3401–3409. <https://doi.org/10.1128/mcb.00006-08>
- Bernard P, Allshire RC (2002) Centromeres become unstuck without heterochromatin. Trends Cell Biol 12(9):419–424. [https://doi.org/10.1016/S0962-8924\(02\)02344-9](https://doi.org/10.1016/S0962-8924(02)02344-9)
- Black BE, Foliz DR, Chakravarthy S, Luger K, Woods VL, Cleveland DW (2004) Structural determinants for generating centromeric chromatin. Nature 430(6999):578–582. <https://doi.org/10.1038/nature02766>
- Blattes R, Monod C, Susbielle G, Cuvier O, Wu JH, Hsieh TS, Laemmli UK, Käs E (2006) Displacement of D1, HP1 and topoisomerase II from satellite heterochromatin by a specific polyamide. EMBO J 25(11):2397–2408. <https://doi.org/10.1038/sj.emboj.7601125>
- Blum JA, Bonaccorsi S, Marzullo M, Palumbo V, Yamashita YM, Barbash DA, Gatti M (2017) The hybrid incompatibility genes Lhr and Hmr are required for sister chromatid detachment during anaphase but not for centromere function. Genetics 207(4):1457–1472. <https://doi.org/10.1534/genetics.117.300390>
- Bobkov GOM, Gilbert N, Heun P (2018) Centromere transcription allows CENP-A to transit from chromatin association to stable incorporation. J Cell Biol 217:1957–1972
- Bosco G, Campbell P, Leiva-Neto JT, Markow TA (2007) Analysis of drosophila species genome size and satellite DNA content reveals significant differences among strains as well as between species. Genetics 177(3):1277–1290. <https://doi.org/10.1534/genetics.107.075069>
- Brajković J, Felicciello I, Bruvo-Madarić B, Ugarković D (2012) Satellite DNA-like elements associated with genes within euchromatin of the beetle *Tribolium castaneum*. G3 Genes Genomes Genetics 2(8):931–941. <https://doi.org/10.1534/g3.112.003467>
- Branco AT, Tao Y, Hartl DL, Lemos B (2013) Natural variation of the Y chromosome suppresses sex ratio distortion and modulates testis-specific gene expression in *Drosophila simulans*. Heredity 111(1):8–15. <https://doi.org/10.1038/hdy.2013.5>
- Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, Hannon GJ (2007) Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. Cell 128(6):1089–1103. <https://doi.org/10.1016/j.cell.2007.01.043>

- Brideau NJ, Barbash DA (2011) Functional conservation of the *Drosophila* hybrid incompatibility gene Lhr. *BMC Evol Biol* 11(1):57. <https://doi.org/10.1186/1471-2148-11-57>
- Brideau NJ, Flores HA, Wang J, Maheshwari S, Wang X, Barbash DA (2006) Two Dobzhansky-Muller genes interact to cause hybrid lethality in *Drosophila*. *Science* 314(5803):1292–1295. <https://doi.org/10.1126/science.1133953>
- Bzymek M, Lovett ST (2001) Instability of repetitive DNA sequences: the role of replication in multiple mechanisms. *Proc Natl Acad Sci USA* 98(15):8319–8325. <https://doi.org/10.1073/pnas.111008398>
- Cabot EL, Doshi P, Wu ML, Wu CI (1993) Population genetics of tandem repeats in centromeric heterochromatin: unequal crossing over and chromosomal divergence at the responder locus of *Drosophila melanogaster*. *Genetics* 135(2):477–487
- Caridi CP, D'agostino C, Ryu T, Zapotoczny G, Delabaere L, Li X, Khodaverdian VY, Amaral N, Lin E, Rau AR, Chiolo I (2018) Nuclear F-actin and myosins drive relocalization of heterochromatic breaks. *Nature* 559(7712):54–60. <https://doi.org/10.1038/s41586-018-0242-8>
- Castillo DM, Barbash DA (2017) Moving speciation genetics forward: modern techniques build on foundational studies in *Drosophila*. *Genetics* 207(3):825–842. <https://doi.org/10.1534/genetics.116.187120>
- Cazemajor M, Joly D, Montchamp-Moreau C (2000) Sex-ratio meiotic drive in *Drosophila simulans* is related to equational nondisjunction of the Y chromosome. *Genetics* 154(1):229–236
- Cernilogar FM, Onorati MC, Kothe GO, Burroughs AM, Parsi KM, Breiling A, Sardo FL, Saxena A, Miyoshi K, Siomi H, Siomi MC, Caminci P, Gilmour DS, Corona DFV, Orlando V (2011) Chromatin-associated RNA interference components contribute to transcriptional regulation in *Drosophila*. *Nature* 480(7377):391–395. <https://doi.org/10.1038/nature10492>
- Chang CH, Chavan A, Palladino J, Wei X, Martins NMC, Santinello B, Chen CC, Erceg J, Beliveau BJ, Wu CT, Larracuente AM, Mellone BG (2019) Islands of retroelements are major components of *Drosophila* centromeres. *PLoS Biol* 17(5). <https://doi.org/10.1371/journal.pbio.3000241>
- Chen CC, Dechassa ML, Bettini E, Ledoux MB, Belisario C, Heun P, Luger K, Mellone BG (2014) CAL1 is the *Drosophila* CENP-A assembly factor. *J Cell Biol* 204(3):313–329. <https://doi.org/10.1083/jcb.201305036>
- Cohen S, Segal D (2009) Extrachromosomal circular DNA in eukaryotes: possible involvement in the plasticity of tandem repeats. *Cytogenet Genome Res* 124(3–4):327–338. <https://doi.org/10.1159/000218136>
- Cohen S, Yacobi K, Segal D (2003) Extrachromosomal circular DNA of tandemly repeated genomic sequences in *Drosophila*. *Genome Res* 13(6):1133–1145. <https://doi.org/10.1101/gr.907603>
- Cohen Z, Bacharach E, Lavi S (2006) Mouse major satellite DNA is prone to eccDNA formation via DNA Ligase IV-dependent pathway. *Oncogene* 25(33):4515–4524. <https://doi.org/10.1038/sj.onc.1209485>
- Copur Ö, Gorchakov A, Finkl K, Kuroda MI, Müller J (2018) Sex-specific phenotypes of histone H4 point mutants establish dosage compensation as the critical function of H4K16 acetylation in *Drosophila*. *Proc Natl Acad Sci USA* 115(52):13336–13341. <https://doi.org/10.1073/pnas.1817274115>
- Courret C, Chang CH, Wei KHC, Montchamp-Moreau C, Larracuente AM (2019) Meiotic drive mechanisms: lessons from *Drosophila*. *Proc R Soc B Biol Sci* 286(1913). <https://doi.org/10.1098/rspb.2019.1430>
- de Lima LG, Hanlon SL, Gerton JL (2020) Origins and evolutionary patterns of the 1.688 satellite DNA family in *Drosophila* phylogeny. *G3 Genes Genomes Genetics* 10(11):4129–4146. <https://doi.org/10.1534/g3.120.401727>
- De Wit E, Greil F, Van Steensel B (2005) Genome-wide HP1 binding in *Drosophila*: developmental plasticity and genomic targeting signals. *Genome Res* 15(9):1265–1273. <https://doi.org/10.1101/gr.3198905>

- deCarvalho AC, Kim H, Poisson LM, Winn ME, Mueller C, Cherba D, Koeman J, Seth S, Protopopov A, Felicella M, Zheng S, Multani A, Jiang Y, Zhang J, Nam D-H, Petricoin EF, Chin L, Mikkelsen T, Verhaak RGW (2018) Discordant inheritance of chromosomal and extrachromosomal DNA elements contributes to dynamic disease evolution in glioblastoma. *Nat Genet* 50(5):708–717. <https://doi.org/10.1038/s41588-018-0105-0>
- Deng X, Meller VH (2006) roX RNAs are required for increased expression of X-linked genes in *Drosophila melanogaster* males. *Genetics* 174(4):1859–1866. <https://doi.org/10.1534/genetics.106.064568>
- Deshpande N, Meller VH (2018) Chromatin that guides dosage compensation is modulated by the siRNA pathway in *Drosophila melanogaster*. *Genetics* 209(4):1085–1097. <https://doi.org/10.1534/genetics.118.301173>
- Dias GB, Heringer P, Svartman M, Kuhn GCS (2015) Helitrons shaping the genomic architecture of *Drosophila*: enrichment of DINE-TR1 in α - and β -heterochromatin, satellite DNA emergence, and piRNA expression. *Chromosom Res* 23(3):597–613. <https://doi.org/10.1007/s10577-015-9480-x>
- Dibartolomeis SM, Tartof KD, Rob Jackson F (1992) A superfamily of *Drosophila* satellite related (SR) DNA repeats restricted to the X chromosome euchromatin. *Nucleic Acids Res* 20(5):1113–1116. <https://doi.org/10.1093/nar/20.5.1113>
- Dorer DR, Henikoff S (1994) Expansions of transgene repeats cause heterochromatin formation and gene silencing in *Drosophila*. *Cell* 77(7):993–1002. [https://doi.org/10.1016/0092-8674\(94\)90439-1](https://doi.org/10.1016/0092-8674(94)90439-1)
- Doshi P, Kaushal S, Benyajati C, Wu C-I (1991) Molecular analysis of the responder satellite DNA in *Drosophila melanogaster*: DNA bending, nucleosome structure, and Rsp-binding proteins. *Mol Biol Evol* 8(5):721–741
- Dover G (1982) Molecular drive: a cohesive mode of species evolution. *Nature* 299:111–117
- Elgin SCR, Reuter G (2013) Position-effect variegation, heterochromatin formation, and gene silencing in *Drosophila*. *Cold Spring Harb Perspect Biol* 5(8):1–26. <https://doi.org/10.1101/cshperspect.a017780>
- Engreitz JM, Pandya-Jones A, McDonel P, Shishkin A, Sirokman K, Surka C, Kadri S, Xing J, Goren A, Lander ES, Plath K, Guttman M (2013) The Xist lncRNA exploits three-dimensional genome architecture to spread across the X chromosome. *Science* 341(6147):1–13. <https://doi.org/10.1126/science.1237973>
- Fackelmayr FO, Dahm K, Renz A, Ramsperger U, Richter A (1994) Nucleic-acid-binding properties of hnRNP-U/SAF-A, a nuclear-matrix protein which binds DNA and RNA in vivo and in vitro. *Eur J Biochem* 221(2):749–757. <https://doi.org/10.1111/j.1432-1033.1994.tb18788.x>
- Fagegaltier D, Bougé AL, Berry B, Poisot É, Sismeiro O, Coppée JY, Théodore L, Voinnet O, Antoniewski C (2009) The endogenous siRNA pathway is involved in heterochromatin formation in *Drosophila*. *Proc Natl Acad Sci USA* 106(50):21258–21263. <https://doi.org/10.1073/pnas.0809208105>
- Fan H, Lv P, Huo X, Wu J, Wang Q, Cheng L, Liu Y, Tang Q-Q, Zhang L, Zhang F, Zheng X, Wu H, Wen B (2018) The nuclear matrix protein HNRNPU maintains 3D genome architecture globally in mouse hepatocytes. *Genome Res* 28(2):192–202. <https://doi.org/10.1101/gr.224576.117>
- Feliciello I, Akrap I, Ugarković Đ (2015) Satellite DNA modulates gene expression in the Beetle *Tribolium castaneum* after heat stress. *PLoS Genet* 11(8):1–18. <https://doi.org/10.1371/journal.pgen.1005466>
- Ferree PM, Barbash DA (2009) Species-specific heterochromatin prevents mitotic chromosome segregation to cause hybrid lethality in *Drosophila*. *PLoS Biol* 7(10). <https://doi.org/10.1371/journal.pbio.1000234>
- Ferree PM, Prasad S (2012) How can satellite DNA divergence cause reproductive isolation? Let us count the chromosomal ways. *Genetics Res Int* 2012:1–11. <https://doi.org/10.1155/2012/430136>

- Fitzgerald DJ, Dryden GL, Bronson EC, Williams JS, Anderson JN (1994) Conserved patterns of bending in satellite and nucleosome positioning DNA. *J Biol Chem* 269(33):21303–21314. [https://doi.org/10.1016/S0021-9258\(17\)31963-4](https://doi.org/10.1016/S0021-9258(17)31963-4)
- Fondon JW, Garner HR (2004) Molecular origins of rapid and continuous morphological evolution. *Proc Natl Acad Sci USA* 101(52):18058–18063. <https://doi.org/10.1073/pnas.0408118101>
- Gallach M (2014) Recurrent turnover of chromosome-specific satellites in *Drosophila*. *Genome Biol Evol* 6(6):1279–1286. <https://doi.org/10.1093/gbe/evu104>
- Garrick D, Fiering S, Martin DIK, Whitelaw E (1998) Repeat-induced gene silencing in mammals. *Nat Genet* 18(1):56–59. <https://doi.org/10.1038/ng0198-56>
- Garrido-Ramos MA (2017) Satellite DNA: an evolving topic. *Genes* 8(9). <https://doi.org/10.3390/genes8090230>
- Gatti M, Pimpinelli S, Santini G (1976) Characterization of *Drosophila* heterochromatin. *Chromosoma* 57(4):351–375. <https://doi.org/10.1007/BF00332160>
- Gell SL, Reenan RA (2013) Mutations to the piRNA pathway component aubergine enhance meiotic drive of segregation distorter in *Drosophila melanogaster*. *Genetics* 193(3):771–784. <https://doi.org/10.1534/genetics.112.147561>
- Goenka A, Sengupta S, Pandey R, Parihar R, Mohanta GC, Mukerji M, Ganesh S (2016) Human satellite-III non-coding RNAs modulate heat-shock-induced transcriptional repression. *J Cell Sci* 129(19):3541–3552. <https://doi.org/10.1242/jcs.189803>
- Göhring F, Fackelmayer FO (1997) The Scaffold/Matrix attachment region binding protein hnRNP-U (SAF-A) is directly bound to chromosomal DNA in vivo: a chemical cross-linking study. *Biochemistry* 36(27):8276–8283. <https://doi.org/10.1021/bi970480f>
- Grewal SIS, Elgin SCR (2007) Transcription and RNA interference in the formation of heterochromatin. *Nature* 447(7143):399–406. <https://doi.org/10.1038/nature05914>
- Gu T, Elgin SCR (2013) Maternal depletion of Piwi, a component of the RNAi system, impacts heterochromatin formation in *Drosophila*. *PLoS Genet* 9(9). <https://doi.org/10.1371/journal.pgen.1003780>
- Hall AC, Ostrowski LA, Mekhail K (2019) Phase separation as a melting pot for DNA repeats. *Trends Genet* 35(8):589–600. <https://doi.org/10.1016/j.tig.2019.05.001>
- Hammock EAD, Young LJ (2005) Genetics: microsatellite instability generates diversity in brain and sociobehavioral traits. *Science* 308(5728):1630–1634. <https://doi.org/10.1126/science.1111427>
- Hartl DL (2000) Molecular melodies in high and low C. *Nat Rev Genet* 1(2):145–149. <https://doi.org/10.1038/35038580>
- Hartl DL, Hartung N (1975) High frequency of one element of segregation distorter in natural populations of *Drosophila melanogaster*. *Evolution* 29(3):512–518
- Helleu Q, Gérard PR, Dubruielle R, Ogereau D, Prud'homme B, Loppin B, Montchamp-Moreau C (2016) Rapid evolution of a Y-chromosome heterochromatin protein underlies sex chromosome meiotic drive. *Proc Natl Acad Sci USA* 113(15):4110–4115. <https://doi.org/10.1073/pnas.1519332113>
- Helleu Q, Courret C, Ogereau D, Burnham KL, Chaminade N, Chakir M, Aulard S, Montchamp-Moreau C (2019) Sex-ratio meiotic drive shapes the evolution of the y chromosome in *Drosophila simulans*. *Mol Biol Evol* 36(12):2668–2681. <https://doi.org/10.1093/molbev/msz160>
- Henikoff S (1998) Conspiracy of silence among repeated transgenes. *BioEssays* 20(7):532–535. [https://doi.org/10.1002/\(SICI\)1521-1878\(199807\)20:7<532::AID-BIES3>3.0.CO;2-M](https://doi.org/10.1002/(SICI)1521-1878(199807)20:7<532::AID-BIES3>3.0.CO;2-M)
- Henikoff S, Ahmad K, Malik HS (2001) The centromere paradox: stable inheritance with rapidly evolving DNA. *Science* 293(5532):1098–1102. <https://doi.org/10.1126/science.1062939>
- Heslop-Harrison JS, Schwarzacher T (2013) Nucleosomes and centromeric DNA packaging. *Proc Natl Acad Sci USA* 110(50):19974–19975. <https://doi.org/10.1073/pnas.1319945110>
- Höck J, Meister G (2008) The Argonaute protein family. *Genome Biol* 9(2). <https://doi.org/10.1186/gb-2008-9-2-210>

- Hsieh T-S, Brutlag D (1979) Sequence and sequence variation within the 1.688 g/cm³ satellite DNA of *Drosophila melanogaster*. *J Mol Biol* 135(2):465–481. [https://doi.org/10.1016/0022-2836\(79\)90447-9](https://doi.org/10.1016/0022-2836(79)90447-9)
- Huertas D, Cortes A, Casanova J, Azorin F (2004) *Drosophila* *DDPI*, a multi-KH-domain protein, contributes to centromeric silencing and chromosome segregation. *Curr Biol* 14:1611–1620
- Huo X, Ji L, Zhang Y, Lv P, Cao X, Wang Q, Yan Z, Dong S, Du D, Zhang F, Wei G, Liu Y, Wen B (2020) The nuclear matrix protein SAFB cooperates with major satellite RNAs to stabilize heterochromatin architecture partially through phase separation. *Mol Cell* 77(2):368–383.e7. <https://doi.org/10.1016/j.molcel.2019.10.001>
- Ideue T, Tani T (2020) Centromeric non-coding RNAs: conservation and diversity in function. *Non-coding RNA* 6(1). <https://doi.org/10.3390/ncrna6010004>
- Iwata-Otsubo A, Dawicki-McKenna JM, Akera T, Falk SJ, Chmátal L, Yang K, Sullivan BA, Schultz RM, Lampson MA, Black BE (2017) Expanded satellite repeats amplify a discrete CENP-A nucleosome assembly site on chromosomes that drive in female meiosis. *Curr Biol* 27(15):2365–2373.e8. <https://doi.org/10.1016/j.cub.2017.06.069>
- Jagannathan M, Warsinger-Pepe N, Watase GJ, Yamashita YM (2017) Comparative analysis of satellite DNA in the *Drosophila melanogaster* species complex. *G3 Genes Genomes Genetics* 7(2):693–704. <https://doi.org/10.1534/g3.116.035352>
- Jagannathan M, Cummings R, Yamashita YM (2019) The modular mechanism of chromocenter formation in *Drosophila*. *BioRxiv*:1–16. <https://doi.org/10.1101/481820>
- Jolly C, Lakhota SC (2006) Human sat III and *Drosophila* hsr ω transcripts: a common paradigm for regulation of nuclear RNA processing in stressed cells. *Nucleic Acids Res* 34(19):5508–5514. <https://doi.org/10.1093/nar/gkl711>
- Joshi S, Meller VH (2017) Satellite repeats identify X chromatin for dosage compensation in *Drosophila melanogaster* males. *Curr Biol* 176(1):139–148. <https://doi.org/10.1016/j.physbeh.2017.03.040>
- Khost DE, Eickbush DG, Larracuent AM (2017) Single-molecule sequencing resolves the detailed structure of complex satellite DNA loci in *Drosophila*. *Genome Res* 27(5):709–721. <https://doi.org/10.1101/gr.213512.116>
- Khurana JS, Xu J, Weng Z, Theurkauf WE (2010) Distinct functions for the *Drosophila* piRNA pathway in genome maintenance and telomere protection. *PLoS Genet* 6:e1001246
- Kim H, Nguyen N-P, Turner K, Wu S, Gujar AD, Luebeck J, Liu J, Deshpande V, Rajkumar U, Namburi S, Amin SB, Yi E, Menghi F, Schulte JH, Henssen AG, Chang HY, Beck CR, Mischel PS, Bafna V, Verhaak RGW (2020) Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers. *Nat Genet* 52(9):891–897. <https://doi.org/10.1038/s41588-020-0678-2>
- Kind J, Vaquerizas JM, Gebhardt P, Gentzel M, Luscombe NM, Bertone P, Akhtar A (2008) Genome-wide analysis reveals MOF as a key regulator of dosage compensation and gene expression in *Drosophila*. *Cell* 133(5):813–828. <https://doi.org/10.1016/j.cell.2008.04.036>
- Kit S (1961) Equilibrium sedimentation in density gradients of DNA preparations from animal tissues. *J Mol Biol* 3(6):711–716. [https://doi.org/10.1016/S0022-2836\(61\)80075-2](https://doi.org/10.1016/S0022-2836(61)80075-2)
- Kiyama R, Matsui H, Oishi M (1986) A repetitive DNA family (Sau3A family) in human chromosomes: extrachromosomal DNA and DNA polymorphism. *Proc Natl Acad Sci* 83(13):4665–4669. <https://doi.org/10.1073/pnas.83.13.4665>
- Kiyama R, Matsui H, Okumura K, Oishi M (1987) A group of repetitive human DNA families that is characterized by extrachromosomal oligomers and restriction-fragment length polymorphism. *J Mol Biol* 193(4):591–597. [https://doi.org/10.1016/0022-2836\(87\)90342-1](https://doi.org/10.1016/0022-2836(87)90342-1)
- Kuhn GCS, Küttler H, Moreira-Filho O, Heslop-Harrison JS (2012) The 1.688 repetitive DNA of *Drosophila*: concerted evolution at different genomic scales and association with genes. *Mol Biol Evol* 29(1):7–11. <https://doi.org/10.1093/molbev/msr173>
- Kuroda MI, Hilfiker A, Lucchesi JC (2016) Dosage compensation in *Drosophila*—a model for the coordinate regulation of transcription. *Genetics* 204(2):435–450. <https://doi.org/10.1534/genetics.115.185108>

- Kursel LE, Malik HS (2018) The cellular mechanisms and consequences of centromere drive. *Curr Opin Cell Biol* 52(206):58–65. <https://doi.org/10.1016/j.ceb.2018.01.011>
- Kusano A, Staber C, Ganetzky B (2001) Nuclear mislocalization of enzymatically active RanGAP causes segregation distortion in *Drosophila*. *Dev Cell* 1(3):351–361. [https://doi.org/10.1016/S1534-5807\(01\)00042-9](https://doi.org/10.1016/S1534-5807(01)00042-9)
- Kusano A, Staber C, Chan HYE, Ganetzky B (2003) Closing the (Ran)GAP on segregation distortion in *Drosophila*. *BioEssays* 25(2):108–115. <https://doi.org/10.1002/bies.10222>
- Larracunte AM (2014) 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* 14(1):1–12. <https://doi.org/10.1186/s12862-014-0233-9>
- Larracunte AM, Presgraves DC (2012) The selfish segregation distorter gene complex of *Drosophila melanogaster*. *Genetics* 192(1):33–53. <https://doi.org/10.1534/genetics.112.141390>
- Larschan E, Bishop EP, Kharchenko PV, Core LJ, Lis JT, Park PJ, Kuroda MI (2011) X chromosome dosage compensation via enhanced transcriptional elongation in *Drosophila*. *Nature* 471(7336):115–118. <https://doi.org/10.1038/nature09757>
- Larson K, Yan S-J, Tsurumi A, Liu J, Zhou J, Gaur K, Guo D, Eickbush TH, Li WX (2012) Heterochromatin formation promotes longevity and represses ribosomal RNA synthesis. *PLoS Genet* 8(1):e1002473. <https://doi.org/10.1371/journal.pgen.1002473>
- Larson AG, Elnatan D, Keenen MM, Trnka MJ, Johnston JB, Burlingame AL, Agard DA, Redding S, Narlikar GJ (2017) Liquid droplet formation by HP1 α suggests a role for phase separation in heterochromatin. *Nature* 547(7662):236–240. <https://doi.org/10.1038/nature22822>
- Levinson G, Gutman G (1987) Slipped-strand mispairing: a major mechanism for DNA sequence evolution. *Mol Biol Evol*. <https://doi.org/10.1093/oxfordjournals.molbev.a040442>
- Lin CJ, Hu F, Dubruille R, Vedanayagam J, Wen J, Smibert P, Loppin B, Lai EC (2018) The hpRNA/RNAi pathway is essential to resolve intragenomic conflict in the *Drosophila* male germline. *Dev Cell* 46(3):316–326.e5. <https://doi.org/10.1016/j.devcel.2018.07.004>
- Lohe AR, Brutlag DL (1987) Identical satellite DNA sequences in sibling species of *Drosophila*. *J Mol Biol* 194:161–170
- Lohe AR, Hilliker AJ, Roberts PA (1993) Mapping simple repeated DNA sequences in heterochromatin of *Drosophila melanogaster*. *Genetics* 134(4):1149–1174. [https://doi.org/10.1016/0168-9525\(93\)90135-5](https://doi.org/10.1016/0168-9525(93)90135-5)
- Lower SS, McGurk MP, Clark A, Barbash DA (2018) Satellite DNA evolution: old ideas, new approaches. *Curr Opin Genet Dev* 49:70–78. <https://doi.org/10.1016/j.gde.2018.03.003>
- Maheshwari S, Barbash DA (2012) Cis-by-trans regulatory divergence causes the asymmetric lethal effects of an ancestral hybrid incompatibility gene. *PLoS Genet* 8(3):e1002597. <https://doi.org/10.1371/journal.pgen.1002597>
- Maison C, Bailly D, Peters AHFM, Quivy J-P, Roche D, Taddei A, Lachner M, Jenuwein T, Almouzni G (2002) Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. *Nat Genet* 30(3):329–334. <https://doi.org/10.1038/ng843>
- Malik HS (2009) The centromere-drive hypothesis: a simple basis for centromere complexity. *Prog Mol Subcell Biol* 48(June):33–52. https://doi.org/10.1007/978-3-642-00182-6_2
- Masumoto H, Masukata H, Muro Y, Nozaki N, Okazaki T (1989) A human centromere antigen (CENP-B) interacts with a short specific sequence in alphoid DNA, a human centromeric satellite. *J Cell Biol* 109(5):1963–1973. <https://doi.org/10.1083/jcb.109.5.1963>
- McDermott SR, Noor MAF (2010) The role of meiotic drive in hybrid male sterility. *Philos Trans R Soc B Biol Sci* 365(1544):1265–1272. <https://doi.org/10.1098/rstb.2009.0264>
- McNulty SM, Sullivan LL, Sullivan BA (2017) Human centromeres produce chromosome-specific and array-specific alpha satellite transcripts that are complexed with CENP-A and CENP-C. *Dev Cell* 42(3):226–240.e6. <https://doi.org/10.1016/j.devcel.2017.07.001>

- Meller VH, Rattner BP (2002) The roX genes encode redundant male-specific lethal transcripts required for targeting of the MSL complex. *EMBO J* 21(5):1084–1091. <https://doi.org/10.1093/emboj/21.5.1084>
- Mendjan S, Taipale M, Kind J, Holz H, Gebhardt P, Schelder M, Vermeulen M, Buscaino A, Duncan K, Mueller J, Wilm M, Stunnenberg HG, Saumweber H, Akhtar A (2006) Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. *Mol Cell* 21(6):811–823. <https://doi.org/10.1016/j.molcel.2006.02.007>
- Menon DU, Meller VH (2012) A role for siRNA in X-chromosome dosage compensation in *Drosophila melanogaster*. *Genetics* 191(3):1023–1028. <https://doi.org/10.1534/genetics.112.140236>
- Menon DU, Coarfa C, Xiao W, Gunaratne PH, Meller VH (2014) siRNAs from an X-linked satellite repeat promote X-chromosome recognition in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 111(46):16460–16465. <https://doi.org/10.1073/pnas.1410534111>
- Meštrović N, Mravinac B, Pavlek M, Vojvoda-Zeljko T, Šatović E, Plohl M (2015) Structural and functional liaisons between transposable elements and satellite DNAs. *Chromosom Res* 23(3):583–596. <https://doi.org/10.1007/s10577-015-9483-7>
- Miga KH, Koren S, Rhie A, Vollger MR, Gershman A, Bzikadze A, Brooks S, Howe E, Porubsky D, Logsdon GA, Schneider VA, Potapova T, Wood J, Chow W, Armstrong J, Fredrickson J, Pak E, Tigyi K, Kremitzki M et al (2020) Telomere-to-telomere assembly of a complete human X chromosome. *Nature* 585(7823):79–84. <https://doi.org/10.1038/s41586-020-2547-7>
- Moschetti R, Caizzi R, Pimpinelli S (1996) Segregation distortion in *Drosophila melanogaster*: genomic organization of responder sequences. *Genetics* 144:1665–1671
- Muchardt C, Guillemé M, Seeler J, Trouche D, Dejean A, Yaniv M (2002) Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1 α . *EMBO Rep* 3(10):975–981. <https://doi.org/10.1093/embo-reports/kvf194>
- Nagao A, Mituyama T, Huang H, Chen D, Siomi MC, Siomi H (2010) Biogenesis pathways of piRNAs loaded onto AGO3 in the *Drosophila* testis. *RNA* 16(12):2503–2515. <https://doi.org/10.1261/rna.2270710>
- Nickerson JA, Krochmalnic G, Wan KM, Penman S (1989) Chromatin architecture and nuclear RNA. *Proc Natl Acad Sci* 86(1):177–181. <https://doi.org/10.1073/pnas.86.1.177>
- Nozawa R-S, Boteva L, Soares DC, Naughton C, Dun AR, Buckle A, Ramsahoye B, Bruton PC, Saleeb RS, Arnedo M, Hill B, Duncan RR, Maciver SK, Gilbert N (2017) SAF-A regulates interphase chromosome structure through oligomerization with chromatin-associated RNAs. *Cell* 169(7):1214–1227.e18. <https://doi.org/10.1016/j.cell.2017.05.029>
- Ohta T, Dover GA (1984) The cohesive population genetics of molecular drive. *Genetics* 108(2):501–521
- Osada N, Innan H (2008) Duplication and gene conversion in the *Drosophila melanogaster* genome. *PLoS Genet* 4(12). <https://doi.org/10.1371/journal.pgen.1000305>
- Pal Bhadra M, Bhadra U, Birchler JA (2006) Misregulation of sex-lethal and disruption of male-specific lethal complex localization in *Drosophila* species hybrids. *Genetics* 174(3):1151–1159. <https://doi.org/10.1534/genetics.106.060541>
- Palomeque T, Lorite P (2008) Satellite DNA in insects: a review. *Heredity* 100(6):564–573. <https://doi.org/10.1038/hdy.2008.24>
- Pardue ML, Gall JG (1970) Chromosomal localization of mouse satellite DNA. *Science* 168(3937):1356–1358. <https://doi.org/10.1126/science.168.3937.1356>
- Peng JC, Karpen GH (2007) H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. *Nat Cell Biol* 9(1):25–35. <https://doi.org/10.1038/ncb1514>
- Petrov DA, Sangster TA, Johnston JS, Hartl DL, Shaw KL (2000) Evidence for DNA loss as a determinant of genome size. *Science* 287(5455):1060–1062. <https://doi.org/10.1126/science.287.5455.1060>
- Phadnis N, Orr HA (2009) Sterility and segregation distortion in *Drosophila* hybrids. *Science* 323(January):376–379

- Piacentini L, Fanti L, Berloco M, Perrini B, Pimpinelli S (2003) Heterochromatin protein 1 (HP1) is associated with induced gene expression in *Drosophila* euchromatin. *J Cell Biol* 161 (4):707–714. <https://doi.org/10.1083/jcb.200303012>
- Pont G, Degroote F, Picard G (1987) Some extrachromosomal circular DNAs from *Drosophila* embryos are homologous to tandemly repeated genes. *J Mol Biol* 195(2):447–451. [https://doi.org/10.1016/0022-2836\(87\)90665-6](https://doi.org/10.1016/0022-2836(87)90665-6)
- Presgraves DC (2010) The molecular evolutionary basis of species formation. *Nat Rev Genet* 11 (3):175–180. <https://doi.org/10.1038/nrg2718>
- Ramírez F, Lingg T, Toscano S, Lam KC, Georgiev P, Chung HR, Lajoie BR, de Wit E, Zhan Y, de Laat W, Dekker J, Manke T, Akhtar A (2015) High-affinity sites form an interaction network to facilitate spreading of the MSL complex across the X chromosome in *Drosophila*. *Mol Cell* 60 (1):146–162. <https://doi.org/10.1016/j.molcel.2015.08.024>
- Ranathunge C, Wheeler GL, Chimahusky ME, Kennedy MM, Morrison JI, Baldwin BS, Perkins AD, Welch ME (2018) Transcriptome profiles of sunflower reveal the potential role of microsatellites in gene expression divergence. *Mol Ecol* 27(5):1188–1199. <https://doi.org/10.1111/mec.14522>
- Richards RI, Sutherland GR (1994) Simple repeat DNA is not replicated simply. *Nat Genet* 6 (2):114–116. <https://doi.org/10.1038/ng0294-114>
- Rizzi N, Denegri M, Chiodi I, Corioni M, Valgardsdottir R, Cobianchi F, Riva S, Biamonti G (2004) Transcriptional activation of a constitutive heterochromatic domain of the human genome in response to heat shock. *Mol Biol Cell* 15(2):543–551. <https://doi.org/10.1091/mbc.e03-07-0487>
- Roach RJ, Garavís M, González C, Jameson GB, Filichev VV, Hale TK (2020) Heterochromatin protein 1 α interacts with parallel RNA and DNA G-quadruplexes. *Nucleic Acids Res* 48 (2):682–693. <https://doi.org/10.1093/nar/gkz1138>
- Rohrbaugh M, Clore A, Davis J, Johnson S, Jones B, Jones K, Kim J, Kithuka B, Lunsford K, Mitchell J, Mott B, Ramos E, Tchedou MR, Acosta G, Araujo M, Cushing S, Duffy G, Graves F, Griffin K et al (2013) Identification and characterization of proteins involved in nuclear organization using *Drosophila* GFP protein trap lines. *PLoS One* 8(1). <https://doi.org/10.1371/journal.pone.0053091>
- Rosin LF, Mellone BG (2017) Centromeres drive a hard bargain. *Trends Genet* 33(2):101–117. <https://doi.org/10.1016/j.tig.2016.12.001>
- Rudd MK, Wray GA, Willard HF (2006) The evolutionary dynamics of α -satellite. *Genome Res* 16 (1):88–96. <https://doi.org/10.1101/gr.3810906>
- Satyaki PRV, Cuykendall TN, Wei KH-C, Brideau NJ, Kwak H, Aruna S, Ferree PM, Ji S, Barbash DA (2014) The Hmr and Lhr hybrid incompatibility genes suppress a broad range of heterochromatic repeats. *PLoS Genet* 10(3):e1004240. <https://doi.org/10.1371/journal.pgen.1004240>
- Sawamura K, Yamamoto MT, Watanabe TK (1993) Hybrid lethal systems in the *Drosophila melanogaster* species complex. II. The Zygotic hybrid rescue (Zhr) gene of *D. melanogaster*. *Genetics* 133(2):307–313
- Sawaya S, Bagshaw A, Buschiazzi E, Kumar P, Chowdhury S, Black MA, Gemmell N (2013) Microsatellite tandem repeats are abundant in human promoters and are associated with regulatory elements. *PLoS One* 8(2). <https://doi.org/10.1371/journal.pone.0054710>
- Schauer T, Ghavi-Helm Y, Sexton T, Albig C, Regnard C, Cavalli G, Furlong EE, Becker PB (2017) Chromosome topology guides the *Drosophila* dosage compensation complex for target gene activation. *EMBO Rep* 18(10):1854–1868. <https://doi.org/10.15252/embr.201744292>
- Schubert T, Pusch MC, Diermeier S, Benes V, Kremmer E, Imhof A, Längst G (2012) Df31 protein and snoRNAs maintain accessible higher-order structures of chromatin. *Mol Cell* 48 (3):434–444. <https://doi.org/10.1016/j.molcel.2012.08.021>
- Schueler MG, Higgins AW, Rudd MK, Gustashaw K, Willard HF (2001) Genomic and genetic definition of a functional human centromere. *Science* 294(5540):109–115. <https://doi.org/10.1126/science.1065042>

- Sharma R, Jost D, Kind J, Gómez-Saldívar G, van Steensel B, Askjaer P, Vaillant C, Meister P (2014) Differential spatial and structural organization of the X chromosome underlies dosage compensation in *C. elegans*. *Genes Dev* 28(23):2591–2596. <https://doi.org/10.1101/gad.248864.114>
- Shatskikh AS, Kotov AA, Adashev VE, Bazylev SS, Olenina LV (2020) Functional significance of satellite DNAs: insights from drosophila. *Front Cell Dev Biol* 8(May):1–19. <https://doi.org/10.3389/fcell.2020.00312>
- Sienski G, Batki J, Senti KA, Dönertas D, Tirian L, Meixner K, Brennecke J (2015) Silencio/CG9754 connects the piwi-piRNA complex to the cellular heterochromatin machinery. *Genes Dev* 29(21):2258–2271. <https://doi.org/10.1101/gad.271908.115>
- Sinclair DA, Guarente L (1997) Extrachromosomal rDNA Circles—a cause of aging in yeast. *Cell* 91(7):1033–1042. [https://doi.org/10.1016/S0092-8674\(00\)80493-6](https://doi.org/10.1016/S0092-8674(00)80493-6)
- Soruco MML, Chery J, Bishop EP, Siggers T, Tolstorukov MY, Leydon AR, Sugden AU, Goebel K, Feng J, Xia P, Vedenko A, Bulyk ML, Park PJ, Larschan E (2013) The CLAMP protein links the MSL complex to the X chromosome during *Drosophila* dosage compensation. *Genes Dev* 27(14):1551–1556. <https://doi.org/10.1101/gad.214585.113>
- Southern EM (1975) Long range periodicities in mouse satellite DNA. *J Mol Biol* 94(1):51–69. [https://doi.org/10.1016/0022-2836\(75\)90404-0](https://doi.org/10.1016/0022-2836(75)90404-0)
- Spieler A, Seum C, Delattre M, Spieler P (2005) Loss of the modifiers of variegation Su(var)3-7 or HP1 impacts male X polytene chromosome morphology and dosage compensation. *J Cell Sci* 118(21):5047–5057. <https://doi.org/10.1242/jcs.02623>
- Spieler A, Begot F, Spieler P, Delattre M (2008) SU(VAR)3-7 links heterochromatin and dosage compensation in drosophila. *PLoS Genet* 4(5):3–7. <https://doi.org/10.1371/journal.pgen.1000066>
- Sproul JS, Khost DE, Eickbush DG, Negm S, Wei X, Wong I, Larracuente AM (2020) Dynamic evolution of euchromatic satellites on the X chromosome in *Drosophila melanogaster* and the simulans Clade. *Mol Biol Evol* 37(8):2241–2256. <https://doi.org/10.1093/molbev/msaa078>
- Straub T, Grimaud C, Gilfillan GD, Mitterweger A, Becker PB (2008) The chromosomal high-affinity binding sites for the *Drosophila* dosage compensation complex. *PLoS Genet* 4(12):1–14. <https://doi.org/10.1371/journal.pgen.1000302>
- Strom AR, Emelyanov AV, Mir M, Fyodorov DV, Darzacq X, Karpen GH (2017) Phase separation drives heterochromatin domain formation. *Nature* 547(7662):241–245. <https://doi.org/10.1038/nature22989>
- Sural TH, Peng S, Li B, Workman JL, Park PJ, Kuroda MI (2008) The MSL3 chromodomain directs a key targeting step for dosage compensation of the *Drosophila melanogaster* X chromosome. *Nat Struct Mol Biol* 15(12):1318–1325. <https://doi.org/10.1038/nsmb.1520>
- Swenson JM, Colmenares SU, Strom AR, Costes SV, Karpen GH (2016) The composition and organization of *Drosophila* heterochromatin are heterogeneous and dynamic. *eLife* 5:1–37. <https://doi.org/10.7554/elife.16096>
- Tao Y, Araripe L, Kingan SB, Ke Y, Xiao H, Hartl DL (2007a) A sex-ratio meiotic drive system in *Drosophila simulans*. II: an X-linked distorter. *PLoS Biol* 5(11):2576–2588. <https://doi.org/10.1371/journal.pbio.0050293>
- Tao Y, Masly JP, Araripe L, Ke Y, Hartl DL (2007b) A sex-ratio meiotic drive system in *Drosophila simulans*. I: an autosomal suppressor. *PLoS Biol* 5(11):2560–2575. <https://doi.org/10.1371/journal.pbio.0050292>
- Tautz D, Trick M, Dover G (1986) Cryptic simplicity in DNA is a major source of genetic variation. *Nature* 322:652–656
- Thomae AW, Schade GOM, Padeken J, Borath M, Vetter I, Kremmer E, Heun P, Imhof A (2013) A pair of centromeric proteins mediates reproductive isolation in drosophila species. *Dev Cell* 27(4):412–424. <https://doi.org/10.1016/j.devcel.2013.10.001>
- Tomilin NV (2008) Regulation of mammalian gene expression by retroelements and non-coding tandem repeats. *BioEssays* 30(4):338–348. <https://doi.org/10.1002/bies.20741>

- Török T, Gorjánác M, Bryant PJ, Kiss I (2000) Prod is a novel DNA-binding protein that binds to the 1.686 g/cm³ 10 bp satellite repeat of *Drosophila melanogaster*. *Nucleic Acids Res* 28 (18):3551–3557. <https://doi.org/10.1093/nar/28.18.3551>
- Turner KM, Deshpande V, Beyter D, Koga T, Rusert J, Lee C, Li B, Arden K, Ren B, Nathanson DA, Kornblum HI, Taylor MD, Kaushal S, Cavenee WK, Wechsler-Reya R, Furnari FB, Vandenberg SR, Rao PN, Wahl GM et al (2017) Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. *Nature* 543(7643):122–125. <https://doi.org/10.1038/nature21356>
- Valgardsdottir R, Chiodi I, Giordano M, Rossi A, Bazzini S, Ghigna C, Riva S, Biamonti G (2008) Transcription of satellite III non-coding RNAs is a general stress response in human cells. *Nucleic Acids Res* 36(2):423–434. <https://doi.org/10.1093/nar/gkm1056>
- Vermaak D, Hayden HS, Henikoff S (2002) Centromere targeting element within the histone fold domain of Cid. *Mol Cell Biol* 22(21):7553–7561. <https://doi.org/10.1128/mcb.22.21.7553-7561.2002>
- Vinces MD, Legendre M, Caldara M, Hagihara M, Verstrepen KJ (2009) Unstable tandem repeats in promoters confer transcriptional evolvability. *Science* 324(5931):1213–1216. <https://doi.org/10.1126/science.1170097>
- Waring GL, Pollack JC (1987) Cloning and characterization of a dispersed, multicopy, X chromosome sequence in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 84(9):2843–2847. <https://doi.org/10.1073/pnas.84.9.2843>
- Wei KHC, Barbash DA (2015) Never settling down: frequent changes in sex chromosomes. *PLoS Biol* 13(4):1–6. <https://doi.org/10.1371/journal.pbio.1002077>
- Wei KHC, Grenier JK, Barbash DA, Clark AG (2014) Correlated variation and population differentiation in satellite DNA abundance among lines of *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 111(52):18793–18798. <https://doi.org/10.1073/pnas.1421951112>
- Willard HF (1985) Chromosome-specific organization of human alpha satellite DNA. *Am J Hum Genet* 37(3):524–532
- Wines DR, Henikoff S (1992) Somatic instability of a drosophila chromosome. *Genetics* 131 (3):683–691
- Wong LH, Brettingham-Moore KH, Chan L, Quach JM, Anderson MA, Northrop EL, Hannan R, Saffery R, Shaw ML, Williams E, Choo KHA (2007) Centromere RNA is a key component for the assembly of nucleoproteins at the nucleolus and centromere. *Genome Res* 17(8):1146–1160. <https://doi.org/10.1101/gr.6022807>
- Wu CI, Lyttle TW, Wu ML, Lin GF (1988) Association between a satellite DNA sequence and the responder of segregation distorter in *D. melanogaster*. *Cell* 54:179–189. [https://doi.org/10.1016/0092-8674\(88\)90550-8](https://doi.org/10.1016/0092-8674(88)90550-8)
- Yanez-Cuna JO, Arnold CD, Stampfel G, Boryń LM, Gerlach D, Rath M, Stark A (2014) Dissection of thousands of cell type-specific enhancers identifies dinucleotide repeat motifs as general enhancer features. *Genome Res* 24(7):1147–1156. <https://doi.org/10.1101/gr.169243.113>
- Yu Q, Colot HV, Kyriacou CP, Hall JC, Rosbash M (1987) Behavior modification by in vitro mutagenesis of a variable region within the period gene of *Drosophila*. *Nature* 326:765–769
- Yuan K, O'Farrell PH (2016) TALE-light imaging reveals maternally guided, H3K9me2/3-independent emergence of functional heterochromatin in *Drosophila* embryos. *Genes Dev* 30 (5):579–593. <https://doi.org/10.1101/gad.272237.115>
- Yunis JJ, Yasminah WG (1971) Heterochromatin, satellite DNA, and cell function. *Science* 174 (4015):1200–1209. <https://doi.org/10.1126/science.174.4015.1200>
- Zhang K, Mosch K, Fischle W, Grewal SIS (2008) Roles of the Clr4 methyltransferase complex in nucleation, spreading and maintenance of heterochromatin. *Nat Struct Mol Biol* 15(4):381–388. <https://doi.org/10.1038/nsmb.1406>