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Principles and functions of pericentromeric satellite DNA clustering into chromocenters

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ABSTRACT

Simple non-coding tandem repeats known as satellite DNA are observed widely across eukaryotes. These repeats occupy vast regions at the centromere and pericentromere of chromosomes but their contribution to cellular function has remained incompletely understood. Here, we review the literature on pericentromeric satellite DNA and discuss its organization and functions across eukaryotic species. We specifically focus on chromocenters, DNA-dense nuclear foci that contain clustered pericentromeric satellite DNA repeats from multiple chromosomes. We first discuss chromocenter formation and the roles that epigenetic modifications, satellite DNA transcripts and sequence-specific satellite DNA-binding play in this process. We then review the newly emerging functions of chromocenters in genome encapsulation, the maintenance of cell fate and speciation. We specifically highlight how the rapid divergence of satellite DNA repeats impacts reproductive isolation between closely related species. Together, we underline the importance of this so-called 'junk DNA' in fundamental biological processes.

1. Introduction

Satellite DNA are simple non-coding tandem repeats that are widespread and abundant in eukaryotic organisms [1]. They are primarily present as vast tracts (10^5 – 10^7 bp) at the centromeric and pericentromeric (flanking the centromere) heterochromatin of eukaryotic chromosomes. Although centromeres are specified through deposition of the centromeric histone [2], the underlying centromeric satellite DNA are also thought to play a role in centromere identity [3,4]. For example, evolutionarily new centromeres (ENCs) and neocentromeres can lack repetitive sequences but still retain the ability to form kinetochores [5–7]. However, the instability of ENCs and neocentromeres [8,9] together with the observation that ENCs 'mature' by acquiring satellite DNA [10] suggests that these repeats are important components of eukaryotic centromeres. In contrast to centromeric satellite DNA, the function (s) of pericentromeric satellite DNA repeats has remained mysterious, even though they far surpass centromeric satellite DNA in abundance. In fact, these sequences are often dismissed as 'junk DNA' [11] or 'selfish parasitic DNA' [12]. However, the widespread occurrence of pericentromeric satellite DNA in eukaryotes suggests that they may serve critical functions that justify their large burden on the cell.

Interestingly, cytological studies have identified that both types of satellite DNA are frequently clustered within nuclei [13–20]. Specifically, pericentromeric satellite DNA from multiple chromosomes are clustered into prominent DNA-dense nuclear foci known as chromocenters [18,19]. Much like for pericentromeric satellite DNA, a concrete biological role for chromocenters has remained elusive. Here, we review the current knowledge on pericentromeric satellite DNA and chromocenters. We focus on recent advances on the mechanisms of pericentromeric satellite DNA clustering into chromocenters and discuss the roles of epigenetic modifications, satellite DNA transcripts and satellite DNA-binding proteins. We also highlight newly discovered functions of pericentromeric satellite DNA that illuminate this underappreciated constituent of eukaryotic genomes.

2. Heterochromatin

The nuclear DNA of eukaryotic organisms is packaged into a nucleoprotein complex known as 'chromatin'. Pioneering work by Emil Heitz identified two classes of chromatin, euchromatin and heterochromatin, based on their differential staining with carmine acetic acid during interphase and mitosis in moss cells [21]. In particular, he used the term

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'heterochromatin' to represent parts of a chromosome that remained intensely stained i.e., condensed, even during interphase. Subsequent research over the past 100 years has identified the structural and functional characteristics of both classes of chromatin. As a result, the molecular mechanisms that mediate the establishment and regulation of euchromatin and heterochromatin have been characterized in fine detail [22]. Euchromatin refers to gene-rich regions on chromosomes that exhibit epigenetic modifications associated with transcriptional activation. In contrast, the gene-poor heterochromatin is often highly condensed throughout all phases of the cell cycle and exhibits epigenetic modifications that are associated with DNA compaction and transcriptional inactivation. Heterochromatin can be further sub-categorized into constitutive and facultative heterochromatin [22,23]. Facultative heterochromatin refers to locus and cell-type specific heterochromatin, e.g. the inactive X chromosome in female mammals. On the other hand, constitutive heterochromatin remains consistently condensed and transcriptionally inactive across different cell types and tissue and is primarily comprised of repetitive sequences such as satellite DNA, telomeres and transposable elements. Importantly, the organization of the genome into a transcriptionally active compartment (euchromatin) and a dense, insert compartment (heterochromatin) is considered to be essential for cell function [23,24].

3. A brief history of satellite DNA

The development of DNA density gradient centrifugation [25] and the measurement of DNA reassociation kinetics [26,27] in the mid-20th century led to the surprising discovery that substantial fractions of eukaryotic genomes were comprised of highly repetitive sequences. The centrifugation of DNA through a dense salt gradient separates DNA molecules of different composition. When density gradient centrifugation was performed on DNA from a variety of eukaryotic species including mouse, guinea pig and crabs, it revealed the presence of 'satellite' bands, which sedimented at different densities compared to the rest of the genome [28–31]. The DNA molecules contained in these 'satellite' bands tended to be AT-rich [30,31] and re-associated with much faster kinetics in comparison to the bulk of genomic DNA [32,33] since they were repetitive and thus able to find a hybridization partner more frequently. These sequences were collectively termed as 'satellite DNA'. Decades of subsequent studies have informed us that satellite DNA are tandem repeats that are present as vast tracts ($10^5 - 10^7$ bp) on eukaryotic chromosomes [32,34,35].

At the same time, the organization of satellite DNA within the nucleus was still enigmatic. The first glimpse into the cellular and chromosomal location of these repetitive sequences was obtained when satellite DNA was found to be associated with purified nucleoli, likely due to the proximity of satellite DNA and the nucleolus organizing rDNA repeats on chromosomes [36]. Subsequent experiments also indicated that satellite DNA repeats were highly abundant in heterochromatin fractions from mouse, guinea pig and other mammals [37–39]. However, the development of in situ hybridization [40,41] was required to unambiguously map the chromosomal locations of these satellite DNA repeats [18,19]. Indeed, in situ hybridization experiments demonstrated for the first time that mouse satellite DNA repeats were present near the centromeres of metaphase chromosomes. Intriguingly, the same experiments revealed that satellite DNA repeats were present in a few DNA-dense foci called chromocenters in interphase nuclei.

4. Chromocenters

The term 'chromocenter' (chromocentri) was originally used by the Italian botanist Pasquale Baccarini and referred to dark stained foci in plant nuclei, which were distinct from nucleoli [42]. It was subsequently noted that chromocenters were largely made up of the heterochromatic regions of chromosomes and often located near nucleoli [21,43]. While satellite DNA repeats are nearly ubiquitous in metazoan chromosomes, chromocenters are most prominently visible in the nuclei of plants, rodents and insects, where they stain intensely with DNA dyes. Pioneering cytological studies using light and electron microscopy in these species have made foundational contributions to our understanding of pericentromeric satellite DNA organization and chromocenter positioning within nuclei [36,44–46]. For example, an analysis of chromocenter spatial positioning in six different mouse cell types revealed that chromocenters are largely present at the nuclear periphery or adjacent to the nucleolus [47]. Notably, only 0.3 – 6% of chromocenters did not adopt these stereotypic nuclear positions in the cell types tested. Thus, the majority of chromocenters are observed either at the nuclear envelope or adjacent to nucleoli. Interestingly, the number of chromocenters per nucleus varied between different mouse cell types. For example, lymphocytes contained the fewest chromocenters per nucleus, while fibroblasts contained the most. The potential functional significance of the number of chromocenters per nucleus will be discussed subsequently.

Following the recent advances in the field [48–50] we propose a more current definition of chromocenters as DNA-dense nuclear organelles that contain pericentromeric satellite DNA from multiple chromosomes (Fig. 1A). Apart from the DNA repeats, chromocenters can also contain satellite RNA [51,52], while sequence-specific DNA-binding proteins [50,53] play a critical role in binding and clustering repeats from multiple chromosomes (Fig. 1A). In eukaryotic nuclei with a 'conventional' organization, chromocenters are either associated with the nuclear envelope or nucleoli or both (Fig. 1B, middle).

In the section below, we highlight the roles played by heterochromatin modifications, satellite DNA transcription and sequence-specific satellite DNA-binding proteins in mediating chromocenter formation. Following that, we discuss current studies, which examine the material properties of chromocenters and suggest that the phenomenon of phase separation plays an important role in satellite DNA clustering into chromocenters. Finally, we review studies that provide a framework to understand the biological function of chromocenters.

5. Factors influencing chromocenter formation

The spatial organization of nuclear compartments has been hypothesized to rely on homotypic interactions between similar sequences, as well as the association of DNA with nuclear scaffolds such as the nuclear envelope (NE) and nucleoli [54–61]. However, the precise mechanisms have remained unclear and the relative contributions of chromatin-chromatin interactions and chromatin-scaffold interactions have been difficult to disentangle. Recently, Solovei, Mirny, Fudenberg and colleagues addressed nuclear compartmentalization in rod photoreceptor nuclei, where chromatin-scaffold interactions are largely absent [62]. These nuclei exhibit a unique 'inverted' organization and typically contain a single large chromocenter in the nuclear interior (Fig. 1B, left) [63]. Using polymer simulations and modulating interaction strengths within and between three chromatin types (euchromatin, facultative heterochromatin and pericentromeric heterochromatin), they were able to reproduce the 'inverted' spatial organization of rod nuclei. Strikingly, their results showed that interactions within pericentromeric heterochromatin (satellite DNA) played a dominant role in bringing about the stereotypic inverted nuclear organization, while interactions involving euchromatin were dispensable [62]. Moreover, the addition of interactions between pericentromeric heterochromatin and the nuclear envelope in the same simulations was sufficient to reproduce conventional nuclear organization, while the separation of euchromatin and heterochromatin into distinct nuclear compartments was still maintained. These data strongly suggest that interactions within pericentromeric heterochromatin i.e. satellite DNA clustering into chromocenters and interactions between chromocenters and the NE/nucleoli are the cornerstones of nuclear spatial organization in eukaryotes.

In this section, we will summarize our current understanding on the factors that drive satellite DNA clustering into chromocenters, including

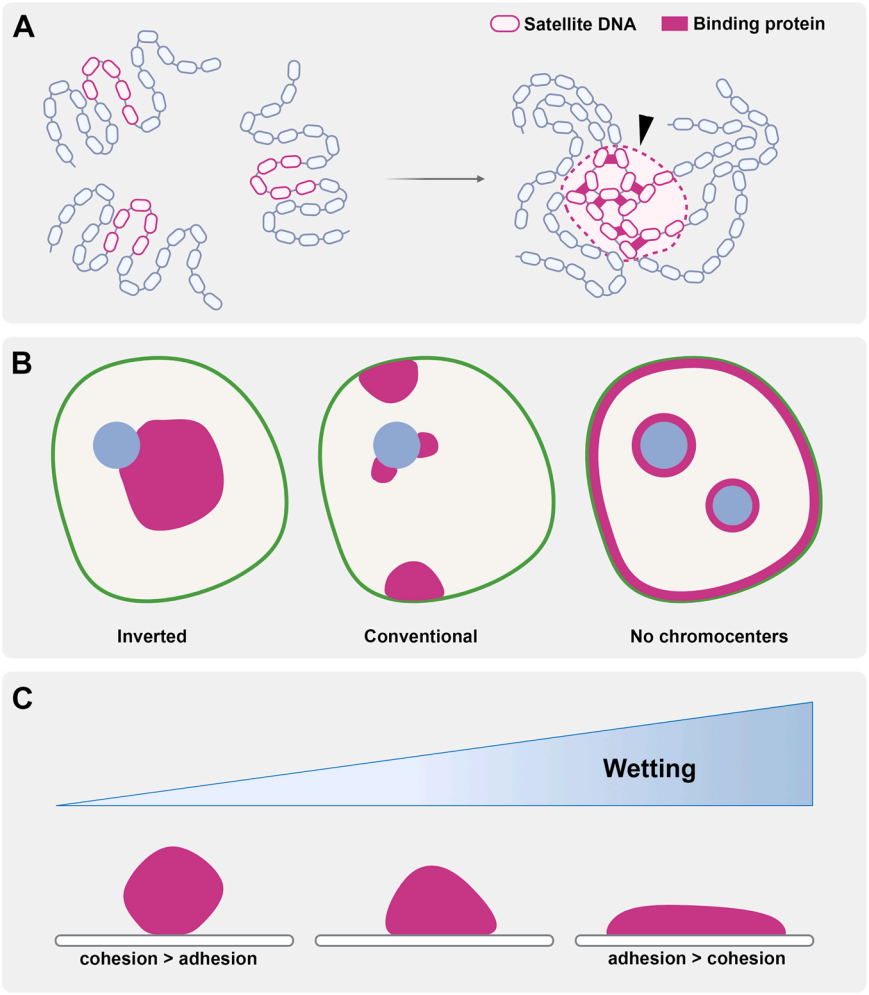

Fig. 1. Models of pericentromeric satellite DNA clustering into chromocenters. (A) A simplified model of how satellite DNA repeats (magenta ovals) on multiple chromosomes can be bound and clustered into a chromocenter (arrowhead) by sequence-specific satellite DNA-binding proteins (magenta rectangles). (B) Pericentromeric satellite DNA organization in three major classes of eukaryotic nuclei. Pericentromeric satellite DNA (magenta), nucleoli (lilac) and the nuclear envelope (green) are depicted in the three classes of nuclei. (C) A theoretical model of how differential 'wetting' of pericentromeric satellite DNA (magenta) against nuclear scaffolds (flat surface) could affect organization within nuclei as depicted in panel B. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

epigenetic modifications on heterochromatin, transcription of satellite DNA repeats and sequence-specific satellite DNA-binding proteins.

5.1. *Recognition of epigenetic modifications on satellite DNA*

5.1.1. *H3K9 methylation and HP1*

Methylation of Histone H3 at K9 is a hallmark of constitutive heterochromatin [64]. Several studies have pointed to the importance of this modification for various aspects of cellular function including genome stability, repeat repression and tethering constitutive heterochromatin to the nuclear lamina [65–71]. Importantly, H3K9 methylation also plays an important role in chromocenter formation. In mice, three paralog pairs of H3 lysine methyltransferases (H3 KMTs) catalyse the methylation of K9 on histone H3 [72]. Specifically, Suv39h1/-Suv39h2 establish H3K9 trimethylation at constitutive heterochromatin [73,74] while Setdb1/Setdb2 are required to repress transposons [75,76] and G9a/Glp mediate gene repression in euchromatin by establishing H3K9 dimethylation [77,78]. Interestingly, the six H3 KMTs display some level of redundancy with one another [79,80] and loss of both Suv39h enzymes does not have a strong effect the morphology of chromocenters. However, a recent study generated mouse embryonic fibroblasts where these KMTs were progressively deleted [72]. The authors noted that cells lacking both Suv39h enzymes as well as Setdb1 lost visible DAPI-dense chromocenters around 40% of the time, in agreement with previous results [80]. Further deletion of all six KMTs led to an even stronger effect with 50–60% of cells exhibiting no chromocenters. Loss of H3K9 methylation was also associated with strong deformations in the nuclear lamina as well as micronuclei formation, both of which can arise from chromocenter and heterochromatin disruption [50,73,81]. Together, these data highlight the importance of H3K9me for pericentromeric satellite DNA clustering into chromocenters.

Epigenetic modifications on chromatin are usually recognized by proteins to elicit specific functional outcomes. A candidate chromocenter forming protein is the heterochromatin-associated protein, HP1, which specifically recognizes trimethylated histone H3K9 [82–84]. Previous studies have shown that the HP1 chromodomain binds H3K9me [82,84] while its chromoshadow domain forms a dimer [85]. Simultaneous DNA-binding and dimerization facilitates HP1 oligomerization, resulting in bridging of H3K9 methylated nucleosomes and compaction of constitutive heterochromatin [86,87]. However, although HP1 is enriched at constitutive heterochromatin in many eukaryotic species, it surprisingly does not play a strong role in promoting the clustering of satellite DNA repeats into chromocenters. Reducing HP1's association with satellite DNA, either through mutation of Suv39h enzymes [73] or expression of a dominant negative HP1 truncation mutant lacking the chromodomain [88] did not affect chromocenter morphology. Rather, acute HP1 depletion appears to decrease nuclear and mitotic chromosome rigidity [89], suggesting that HP1 affects the stiffness of chromatin through nucleosome bridging. Intriguingly, these studies also suggest that the proteins which recognize H3K9me and mediate satellite DNA clustering into chromocenters are still undiscovered.

5.1.2. *Cytosine methylation and MeCP2*

Another set of potential chromocenter forming proteins are MeCP2 and MBD2, methyl-CpG binding domain (MBD) proteins that bind 5-

methyl cytosine and are enriched at chromocenters [90,91]. Notably, mutations in MeCP2 are associated with Rett syndrome, a common neurodevelopmental disorder [92,93]. The strongest evidence linking MeCP2 and MBD2 to chromocenter formation comes from experiments where both proteins were ectopically expressed in mouse myoblast culture cells, which resulted in fewer chromocenters (i.e. increase in satellite DNA clustering) [94]. The ability of MeCP2 to cluster satellite DNA relied solely on the methyl binding domain and was functional even in the absence of H3K9 methylation. In contrast, ectopic expression of HP1a or the centromeric protein CENPB in the same context did not alter satellite DNA clustering or chromocenter numbers per nucleus [94]. Live imaging in mouse cells also revealed multiple instances of chromocenter fusion during interphase upon MeCP2 overexpression. Moreover, in vitro experiments have shown that MeCP2 can interconnect nucleosome arrays into oligomers, suggesting a possible mechanism for satellite DNA clustering [95]. At the same time, it should be noted that MeCP2-mutant mice did not exhibit any gross defects in chromocenter formation [94]. It remains to be tested whether other MBD proteins may function redundantly with MeCP2 to mediate chromocenter formation, in a similar manner to H3 KMTs. In addition, several observations suggest that other mechanisms may play a larger role in satellite DNA clustering into chromocenters: low levels of cytosine methylation in mice [96], the absence of cytosine methylation in *Drosophila* [97] and the weak correlation between cytosine methylation and chromocenter morphology in plants and mice [98,99].

5.2. Satellite DNA transcription

Multiple studies have also identified that chromocenters are regulated by satellite DNA transcripts. For example, a burst of satellite DNA transcription is essential for chromocenter establishment in the early mouse embryo soon after fertilization [52]. Blocking satellite DNA transcription in the early embryo by injecting LNA:DNA gapmers resulted in developmental arrest at the 2-cell stage prior to chromocenter formation [52,100]. More recent work has identified that nascent satellite DNA transcripts remain associated with chromocenters and help recruit the Suv39h KMTs to satellite DNA [101–103]. These studies therefore link satellite DNA transcripts to the maintenance of H3K9 methylation at chromocenters. These data are reminiscent of studies from plants, insects and fungi, where pericentric transcripts are processed into small RNAs and mediate heterochromatin formation and maintenance [22,104–106]. However, studies on satellite RNA regulation of mouse chromocenters paint a subtly different picture. Here, nascent satellite DNA transcripts are thought to hybridize with the associated DNA repeat and the resulting satellite RNA:DNA hybrid serves as a scaffold for binding proteins [101]. Moreover, the satellite DNA transcripts are decorated with the m6A RNA modification, which is thought to play a role in RNA:DNA hybrid formation and the retention of RNA on heterochromatin [107]. Despite the presence of satellite RNA:DNA hybrids at chromocenters, blocking their transcription in cultured mouse cells does not strongly affect satellite DNA clustering [108]. On the other hand, increasing satellite DNA transcription by gRNA-targeting of dCas9-VPR in mouse cells [109] resulted in the loss of satellite DNA clustering. However, the dramatic breakdown of chromocenters observed in this study is unlikely to depend solely on satellite DNA transcription; a previous study has demonstrated that upregulation of satellite DNA transcripts (as a result of transcriptional repressor depletion) did not affect chromocenters to the same degree [110]. Rather, work from Erdel, Rippe and colleagues suggests that satellite DNA transcription and eviction of other resident satellite DNA-associated proteins by dCas9-VPR may function cooperatively to inhibit chromocenter formation [109].

5.3. Sequence-specific satellite DNA-binding proteins

The studies described above characterize epigenetic mechanisms that mediate pericentromeric satellite DNA clustering into chromocenters. However, these epigenetic modifications (H3K9me, cytosine methylation) are also observed in other chromosomal locations, outside of pericentromeric heterochromatin. Therefore, how pericentromeric satellite DNA repeats are selectively incorporated into chromocenters has remained incompletely understood. We propose that this gap in our knowledge can be filled by sequence-specific satellite DNA-binding proteins, an overlooked class of chromocenter-forming proteins. Two proteins, mammalian HMGA1 (formerly HMG-1/Y) and D1 in *Drosophila* were initially identified as abundant non-histone chromosomal proteins [111–114]. D1 was demonstrated to strongly associate with two abundant AT-rich satellite DNA (the 359 bp repeat and the 5 bp AATAT repeat) in *Drosophila* [112,115–117], while HMGA1 was shown to bind three sites on the 172 bp α-satellite of the African green monkey [111] and the pericentromeric mouse major satellite[118]. Subsequently, the proliferation disruptor (Prod) protein from *Drosophila* was identified to specifically bind the abundant 10 bp AATAACATAG repeat [119]. What distinguishes HMGA1, D1 and Prod from other heterochromatin-associated proteins is that all three proteins have been demonstrated to cluster satellite DNA into chromocenters [50,53]. While HMGA1 has also been associated with several other cellular functions [120,121], we will focus here on the studies of HMGA1, D1 and Prod that highlight their importance in chromocenter function.

Both HMGA1 (3 AT-hooks) and D1 (11 AT-hooks) contain multiple DNA-binding motifs known as AT-hooks. AT-hooks are short arginine- and proline-rich sequences that bind the minor groove of AT-rich DNA in a sequence-specific manner and likely recognize specific secondary structures [122,123]. Footprinting assays of HMGA1 binding sites on the α-satellite and SV40 DNA demonstrated that the HMGA1 AT-hooks bind runs of 5–6 A/T base pairs [111,124]. On the α-satellite, HMGA1 binds three distinct sites, which could theoretically be brought into proximity when the 172 bp satellite DNA monomer is wrapped around the nucleosome [111]. This binding pattern has been proposed to facilitate the regular positioning of nucleosomes at satellite DNA repeats. Interestingly, multiple studies of *Drosophila* constitutive heterochromatin have observed regularly spaced nucleosomes [66,125,126].

Strikingly, electron microscopy of linearized plasmid DNA containing the mouse pericentromeric major satellite DNA incubated with purified HMGA1 suggested that HMGA1 could effectively crosslink distinct DNA strands [127]. Moreover, all three HMGA1 AT-hooks were required for effective crosslinking, suggesting that a single HMGA1 molecule may contact more than one DNA strand through its multivalent DNA-binding motifs. Subsequently, studies in cultured cells have shown that depletion of HMGA1 in multiple mouse cell lines disrupted chromocenters [50,128]. Specifically, 30–40% of HMGA1 depleted cells contained nuclei with dispersed thread-like satellite DNA and disrupted chromocenters [50]. Taken together, these data suggest that mouse major satellite DNA repeats are clustered into chromocenters through the partially redundant functions of HMGA1, proteins that binds methylated H3K9, and MeCP2 (which contains 2 AT-hook motifs and binds methylated cytosines adjacent to A/T sequences) [129,130]. Consistently, HMGA1 remains localized to satellite DNA in the few H3K9 methylation deficient cells with intact chromocenters [72].

Studies have also revealed that loss-of-function mutations of the *Drosophila* D1 and Prod satellite DNA-binding proteins result in disrupted chromocenters [50,53]. Importantly, these studies showed that D1 and Prod were sufficient to cluster pericentromeric satellite DNA. First, tethering D1 to 10 kb LacO arrays (present in genomic loci that are distant from pericentromeric heterochromatin) resulted in the recruitment of these LacO arrays to *Drosophila* chromocenters [50]. Second, ectopic expression of Prod in *Drosophila* spermatocytes, where the cognate AATAACATAG loci are spatially separated, was sufficient to link the AATAACATAG loci across the nucleus through satellite DNA-containing proteinaceous threads [53]. Third, *Drosophila* D1 strikingly localized to chromocenters when expressed in mouse cells, despite nearly 600 million years of evolution separating these organisms

[50]. In mouse cells, increased expression of D1 resulted in fewer and larger chromocenters per nucleus, suggesting that D1 can effectively cluster satellite DNA from multiple chromosomes, even in a foreign context.

Taken together, these studies highlight the ability of sequence-specific DNA-binding proteins to bind pericentromeric satellite DNA from multiple chromosomes and cluster them into chromocenters (Fig. 1A).

6. The material properties of chromocenters and heterochromatin

Eukaryotic cells are characterized by the compartmentalization of various biological processes into organelles [131]. While some organelles, such as the nucleus and mitochondria, are enclosed by membranes, other cellular components such as P-granules, nucleoli and balbiani bodies, exhibit organelle-like compartmentalization without enclosing membranes [131,132]. The compartmentalization of these membrane-less organelles is thought to rely on the process of liquid-liquid phase separation (LLPS). During LLPS, biomolecules like DNA or proteins in solution thermodynamically disfavour interactions with the solvent upon reaching a concentration threshold (C_{sat}) [133]. A further increase in the concentration of the molecule then results in a separation of the solution into distinct liquid phases [133,134], visually reminiscent of oil droplets in a bowl of soup. The 'dense' phase where the protein or nucleic acid is concentrated is frequently referred to as a biomolecular condensate. LLPS is influenced by multiple features, including intrinsically disordered regions (IDRs) on proteins, the capacity for multivalent interactions (protein-protein or protein-nucleic acid) and the cellular environment (pH, temperature) [132,135].

6.1. Phase separation of chromocenters

6.1.1. Chromocenters as a liquid droplet

Accumulating evidence suggests that LLPS plays a role in the compartmentalization of chromocenters, which also lack enclosing membranes. As often observed with other phase separated organelles, chromocenters frequently fuse with one other [63,94]. Chromocenter fusion occurs naturally during terminal differentiation of multiple cell types, as well as under exogenous conditions such as overexpression of MeCP2 and D1 [50,94]. Moreover, chromocenter fusion intermediates often resemble the 'hourglass' shape typified by liquid-like droplets. In addition, chromocenter-associated proteins exhibit many of the characteristics that are required for LLPS. First, they typically possess the ability to bind multiple strands of DNA through multivalent DNA-binding domains [111,112,129,136]. Second, they contain intrinsically disordered regions, which can promote LLPS [137,138]. Consistently, chromocenter-associated proteins form liquid-like droplets in vitro, either alone [137,139,140] or in complex with DNA [141–143]. Third, fluorescence recovery after photobleaching (FRAP) experiments show that some of these proteins exhibit dynamic binding to the chromatin substrate in vivo [143–145]. Such rapid molecular exchanges are frequently observed in liquid-like compartments. Fourth, *Drosophila* chromocenters (marked by HP1) exhibit liquid-like properties during heterochromatin establishment in early embryonic cycles [139]. Together, these characteristics favour LLPS as an organizing principle for chromocenters. Moreover, the liquid-like properties of chromocenters likely play a significant role in the diversity of heterochromatin spatial organization across cells and tissues.

6.1.2. Chromocenters as a chromatin globule

Interestingly, a recent study challenges the idea that chromocenters possess liquid-like properties. Using HP1 as a candidate phase separating protein, Erdel, Rippe and colleagues have proposed that mouse chromocenters may exist as a chromatin globule rather than a liquid droplet [109]. In their model, they suggest that chromatin bridging and not interactions between intrinsically disordered regions is largely responsible for chromocenter formation. They propose a 'chromatin globule' model, which predicts that the size of chromocenters is kept constant, even in response to changes in protein concentration. This marks a key difference from a condensate that forms via LLPS, where the size of a droplet will immediately react to changes in concentration. Consistently, they observed that changes in HP1 concentration did not alter chromocenter size or number, which has also been observed in previous studies [73,94]. In principle, both the 'chromatin globule' model and the 'liquid droplet' model are compatible with the large-scale re-organization and fusions of chromocenters that occur during terminal differentiation, even though the kinetics of chromocenter fusions are likely to be different in these two cases. However, it should be noted that the above study dissects the material properties of chromocenters in relation to HP1 protein levels. We suggest that it would also be important to analyse chromocenter material properties in cultured mouse cells when over-expressing proteins that have been previously demonstrated to elicit changes in chromocenter size and number [50,94]. One possibility is that that chromocenters may exist stably as chromatin globules with transient switches to a droplet-like state during fusion and large-scale re-organization.

6.1.3. Solid-like or liquid-like heterochromatin?

The precise material properties of heterochromatin in cells are also presently debated. Prior in vitro analyses have shown that chromatin can form reversible condensates by altering salt concentrations [146,147]. These in vitro chromatin condensates are considered to have undergone a liquid-solid phase separation (LSPS). A recent paper by Hendzel, Hansen and colleagues suggests that chromatin, and specifically chromocenter-associated heterochromatin, exhibits solid-like properties in vitro and in vivo [148]. Relying largely on FRAP experiments, they observed that native chromatin preparations and in vitro assembled nucleosome arrays show very limited molecular exchange and solid-like properties. Remarkably, the solid-like chromatin state was also observed at mouse chromocenters in vivo and co-existed with the more rapidly exchanging heterochromatin proteins, HP1 and KMT5C [148]. In contrast, work from Rosen and colleagues (and subsequently verified by Hendzel, Hansen and colleagues) reported that in vitro assembled nucleosome arrays can exhibit liquid-like properties under specific conditions [148,149]. Briefly, the liquid-like properties of these nucleosome arrays arise when histone tail-DNA interactions are weakened, thereby allowing increased molecular exchange. Rosen and colleagues also observed that spacing nucleosomes with gaps of 10 n + 5-bp promoted phase separation, while the addition of the linker histone H1 increased droplet density but slowed down dynamics (less liquid-like droplets) [149]. Interestingly, HMGA1 has been demonstrated to evict Histone H1 from AT-rich regions of the genome [150] and has also been proposed to position nucleosomes on satellite DNA repeats. As such, HMGA1 may promote liquid-like properties locally when bound to satellite DNA-containing chromatin.

6.2. A model for the absence of chromocenters in certain cells

Phase separation may also provide an explanation for the absence of visible chromocenters in certain organisms (Fig. 1B, right). For instance, human cells exhibit almost no DAPI-dense nuclear foci even though a substantial portion of the human genome consists of AT-rich satellite DNA repeats [20,151,152]. We propose that this paradox can be explained when considering chromocenters as a liquid-like droplets with different wetting properties in different cell types. Wetting refers to the ability of a liquid to spread over a solid surface; cohesive forces within the liquid limit wetting, while adhesive forces between the liquid and the solid surface promote wetting (Fig. 1C). We propose that satellite DNA-binding proteins and other chromocenter-associated proteins represent the 'cohesive' forces within chromocenters, while interactions between satellite DNA-containing heterochromatin and nuclear

scaffolds such as the nuclear envelope (NE) or nucleolus represent the 'adhesive' forces. A balance between the cohesive and adhesive forces can be found in chromocenter-containing cells with a 'conventional' nuclear organization (Fig. 1B, middle). When the cohesive forces predominate, an 'inverted' nuclear organization is expected and can be observed in cells such as mouse rod photoreceptors, which do not express the heterochromatin-NE tethering proteins, LBR and Lamin A (Fig. 1B, left) [63,153]. On the other end of the spectrum, we propose that dominant adhesive forces in human cells i.e. extensive interactions between satellite DNA-containing heterochromatin and nuclear scaffolds, should lead to few, if any, visible chromocenters (Fig. 1B, right). One prediction from our hypothesis is that a change in the balance of these adhesive and cohesive forces in human cells could lead to DAPI-dense nuclear foci. An instance of this may be occurring in senescent human cells, which can exhibit dramatic DAPI-dense senescence-associated heterochromatic foci (SAHF) [154]. Consistent with our idea, senescent human cells decrease Lamin B expression (decreased adhesion) [155–157], while concurrently increasing levels of SAHF-associated HMGA1 (increased cohesion) [154]. While our model requires further investigation, it predicts that human nuclei may still contain clustered satellite DNA, but just not as DNA-dense chromocenters. Rather, clustered pericentromeric repeats may be spread out over the surface of nuclear scaffolds like the NE and the nucleolus. In accordance with this notion, a previous study has shown that human cells exhibit higher-order folding of pericentromeric satellite DNA repeats [158]. Importantly, the potential clustering of human chromosomes through pericentromeric satellite DNA may still facilitate the important biological functions attributed to chromocenters.

7. Biological functions of chromocenters

Satellite DNA repeats can comprise between 5% and 50% of eukaryotic genomes and organisms must utilize a significant fraction of their biosynthetic capacity to replicate and segregate these supposedly non-functional repeats [1]. We think that it is unlikely that organisms carry a tremendous amount of satellite DNA that is either passive or detrimental to their existence. Rather, we find it more likely that these repeats have been co-opted to perform important cellular functions. Consistent with this possibility, the presence of identical repeats across the genome could function to structure or physically link different chromosomes through a specialized protein machinery. Based on this reasoning, researchers have proposed a variety of roles for satellite DNA repeats [159,160]. In this section, we will summarize functions of pericentromeric satellite DNA that are linked to their ability to cluster into chromocenters, specifically genome encapsulation and regulation of gene expression and cell fate.

7.1. The role of chromocenters in genome encapsulation

The presence of a single nucleus encapsulating the entire genome is a defining feature of eukaryotes. However, eukaryotic genomes are typically split into multiple chromosomes, which poses a significant logistical challenge to genome encapsulation. Moreover, genome encapsulation must be maintained throughout the cell cycle, especially during cell division, when the nucleus is typically broken down in the mother cell and re-assembled in the daughter cells. Multiple studies have identified a variety of mechanisms that ensure genome encapsulation during and following mitosis [161–165]. A failure to correctly encapsulate the genome often results in the formation of micronuclei, chromosomes or chromosomal fragments that are isolated from the primary nucleus [166,167]. Micronuclei are particularly deleterious for genomic stability and cell viability and their presence is often associated with disease states [168].

Interestingly, recent work has identified a novel function for chromocenters and pericentromeric satellite DNA in ensuring genome encapsulation during interphase [50,53]. These studies used depletion of sequence-specific satellite DNA-binding proteins to elicit targeted disruption of chromocenters in both *Drosophila* tissues and mouse cells. Mutation of the D1 and Prod satellite DNA-binding proteins in *Drosophila*, which cluster the AATAT and AATAACATAG satellite DNA repeats respectively, and depletion of HMGA1 in cultured mouse cells, resulted in a significant increase of cells containing micronuclei. Notably, these MN were not associated with mitotic errors. Rather, live imaging revealed that these MN formed during interphase by budding out of the primary nucleus. In addition, the MN largely contained the satellite DNA repeats whose clustering was disrupted. For instance, ~80% of micronuclei in prod mutant tissues contained the AATAACATAG repeat [53]. Together, these studies suggest that chromocenters and pericentromeric satellite DNA physically link the entire chromosome complement and maintain the genome in a single nucleus. When chromocenters are disrupted, de-clustered chromosomes can bud out of the interphase nucleus forming micronuclei and triggering DNA damage and cell death [50,53].

Most eukaryotic genomes (with the notable exception of mouse) contain multiple unique pericentromeric satellite DNA repeats. For example, the *Drosophila melanogaster* genome contains at least 19 unique satellite DNA repeats, which are non-uniformly distributed across the chromosomes [169–172]. This is exemplified by the AATAT repeat, which is present abundantly on the 4th and Y chromosome, at lower levels on the 3rd and X chromosome and completely absent on the 2nd chromosome. Similarly, the AATAACATAG repeat is only present on the 2nd and 3rd chromosomes. Within the *D. melanogaster* nucleus, the AATAT-binding protein D1 and the AATAACATAG-binding protein Prod form multiple foci rather than a single chromocenter [53]. This raises the question of how multiple satellite DNA repeats and their cognate binding proteins promote the encapsulation of the entire genome into a single nucleus. In *Drosophila*, this could be facilitated by the association of D1 and Prod within the broader (HP1-positive) heterochromatin compartment. Surprisingly, this association does not seem to result from direct protein-protein interactions [53]. Rather, we propose that the D1-Prod associations can be mediated by the physical proximity of repeats bound by both proteins. For example, the *D. melanogaster* 3rd chromosome contains both the AATAT and the AATAACATAG repeats and could function as a platform to bring both D1-containing and Prod-containing chromocenters into association. This can be illustrated in a simplified model of three chromosomes, where two satellite DNA-repeats are non-uniformly distributed (Fig. 2A). In this model, the presence of more than one repeat on a chromosome (arrowhead) allows it to cluster with the other two chromosomes, which each contain a single unique satellite DNA repeat. If this model is correct, chromosomes carrying more than one type of satellite DNA repeat, as is often observed in eukaryotes [160], could function as platforms to link multiple chromosomes. Such a process occurring simultaneously on multiple chromosomes could effectively network the entire chromosome complement, thus promoting the maintenance of the genome in a single nucleus.

One of the most interesting questions that remains to be addressed is how de-clustered chromosomes 'bud' out of the interphase nucleus to form MN. Recurrent breaches of the nuclear envelope (NE) that are observed following chromocenter disruption highlight one possible route out of the nucleus for de-clustered chromosomes [50]. However, the relationship between chromocenters and nuclear integrity still remains poorly understood. Excitingly, recent work has highlighted an important role for heterochromatin in nuclear integrity [173]. These studies have demonstrated that chromatin as well as the nuclear lamina play complementary roles in promoting the mechanical rigidity of nuclei [81].

While the nuclear lamina functions primarily in resisting large deforming forces on the nucleus, chromatin primarily responds to small deforming forces [174]. Consistently, increasing heterochromatin levels (enhanced chromatin compaction) led to nuclear stiffening and improved resistance to small deformations [175]. Moreover, increased

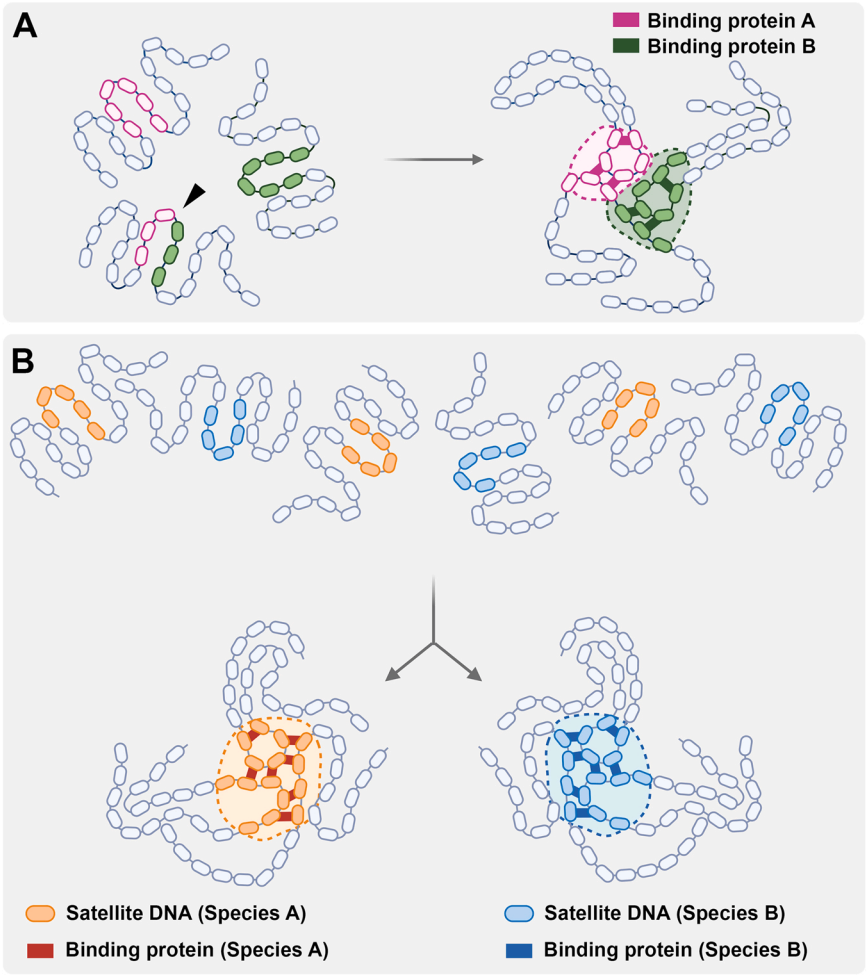

Fig. 2. Models of chromocenter formation from multiple pericentromeric satellite DNA repeats. (A) A simplified model of how a chromosome carrying two satellite DNA repeats (arrowhead, different repeats are represented by magenta ovals and green ovals) can link two other chromosomes carrying unique satellite DNA repeats through the action of cognate satellite DNA-binding proteins (magenta and green rectangles) (B) A model of how co-evolved repeats (orange ovals, blue ovals) and binding proteins (dark orange rectangles, dark blue rectangles) can facilitate clustering of specific chromosomes into chromocenters and promote recognition of 'self' DNA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

heterochromatin levels can also rescue nuclear morphology in a cell line that models the Hutchinson-Gilford progeria syndrome, a disease associated with Lamin A mutation [175]. Thus, Stephens and colleagues propose that chromatin is the main structural element of the nucleoplasm, while the lamin network at the nuclear periphery acts as a "flexible yet strong bag" that is influenced by the chromatin [175]. In this context, it is important to note that heterochromatin-associated proteins and heterochromatin-NE association can have striking effects on chromatin organization [62], thereby affecting the structural and mechanical properties of the nucleus. Further work is thus required to better understand whether chromocenter disruption alters nuclear rigidity and how the de-clustering of chromosomes may contribute to the observed loss of nuclear integrity.

7.2. *Chromocenters, gene expression and cell fate*

Even though the total satellite DNA content per cell remains the same throughout an organism, different cell types can contain vastly different numbers of chromocenter per nucleus. Interestingly, multiple studies have suggested that changes in the number of chromocenters per nucleus is associated with changes in cell fate. Variation in the number of chromocenters per nucleus was first reported in the brains of mice, guinea pigs and hamsters where specific postmitotic neuronal cell types exhibited fewer, but larger, chromocenters than other cells [46,176,177]. These initial observations were followed up in Purkinje cells, which exhibited a strong reduction in the number of chromocenters during early mouse postnatal development [13]. While ~8 chromocenters per nucleus are observed at P0, this number is reduced by the first week of postnatal development to ~5 per nucleus, with one or two large chromocenters positioned around a central nucleolus. By the second and third week of post-natal development, an increase in the number of chromocenters per nucleus is observed with these smaller chromocenters redistributed to the nuclear periphery. Interestingly, this reorganization of chromocenters between the first and third postnatal week is associated with the growth of Purkinje cell dendrite trees, synapse formation and terminal differentiation [13]. Another more recent example linking chromocenters to cell fate can be found in olfactory sensory neurons (OSNs) [178]. Each of these cells expresses a single olfactory receptor (OR) from a choice of ~2800 ORs. In the mouse genome, these 2800 ORs are present in 92 gene clusters that are enriched for AT-rich DNA. Using a panOR FISH probe, Lomvardas and colleagues demonstrated that all inactive ORs are located in ~5 clusters around a central chromocenter with the important exception of the single active OR, which is present in the nuclear interior [178]. Ectopic expression of the NE-tethering Lamin B receptor (LBR) in OSNs resulted in recruitment of satellite DNA to the nuclear periphery as well as chromocenter dispersal [178]. These changes in satellite DNA organization led to perturbed OR clustering and the loss of cell-specific OR expression in OSNs. In addition, a decrease in the number of chromocenter per nucleus has also been observed during the transition of mouse myoblasts into terminally differentiated myotubes [94] as well as during terminal differentiation in other cell lineages and species [179–183]. Together, these studies suggest that changes in satellite DNA organization can influence terminal differentiation and the maintenance of cell fate.

In general, the changes in satellite DNA organization are thought to

influence differentiation and cell fate through gene regulation. This was first shown in studies on the Ikaros proteins, which are sequence-specific transcription factors that have a profound effect on lymphoid development [184]. Interestingly, Ikaros proteins were found to localize to chromocenters and were frequently associated with repressed but not with actively expressed gene loci [185–187]. Further experiments showed that the recruitment of genes to chromocenters by Ikaros and their subsequent repression likely results in a lymphocyte-specific gene expression program [186]. This raises the question as to how specific genes or loci are recruited to chromocenters. One possible mechanism may involve short tracts of repetitive DNA, sometimes referred to as microsatellites, that are interspersed on the euchromatic arms of eukaryotic chromosomes [188,189]. It is conceivable that these microsatellite loci engage in homotypic interactions with identical repeats at chromocenters, thereby influencing the expression of microsatellite-proximal genes. An example of this phenomenon is observed in the bw^D *Drosophila* strain, in which a large tract of the AAGAG pericentromeric satellite DNA repeat is translocated to euchromatin, in proximity to the eye pigment gene brown. Here, homotypic interactions between the bw-proximal AAGAG repeats and pericentromeric AAGAG repeats result in the long-range looping of the bw gene to the chromocenter, where it is strongly repressed [190]. This results in reduced eye pigment (only ~5% of the eye cells are pigmented) in bw^D flies [191]. What remains to be determined is how many copies of a repeat are required to mediate such long-range interactions between microsatellite loci in euchromatin and the chromocenter. Moreover, the proteins that directly mediate homotypic interactions between euchromatic satellite DNA repeats and the chromocenter are yet to be conclusively identified, although some candidate proteins have been proposed [192]. Further studies are also required to conclusively address how the number of chromocenters per nucleus affects cell-specific gene expression program, differentiation and cell fate.

A unique role for changes in satellite DNA organization, independent of gene expression regulation, is observed in the rod photoreceptor cells of nocturnal mammals [63]. In these cells, nuclei adopt an 'inverted' heterochromatin organization (Fig. 1B, left) where a singular massive chromocenter is surrounded by concentric shells of facultative heterochromatin and euchromatin. The inversion of rod photoreceptor nuclei occurs during the course of postnatal development; rod nuclei at P0 exhibit a conventional organization (chromocenters tethered at the nucleolus and nuclear envelope) while rod nuclei from 9 months old mice exhibit a completely inverted organization. Further experiments showed that the inverted organization relies upon the loss of expression of the NE-tethering genes, LBR and Lamin A/C in rod nuclei [153]. Indeed, the simultaneous loss of LBR and Lamin A/C resulted in the ectopic formation of inverted nuclei in all differentiated cell types of newborn mice [153]. Surprisingly, the inverted nuclear organization in rod cells is not thought to affect gene expression. Rather, the central concentration of heterochromatin in rod nuclei is thought to improve the transmission of light to the light-sensing region of the retina, thereby aiding the vision of nocturnal animals [63].

8. Satellite DNA divergence and reproductive isolation between species

The biological species concept (BSC) defines species as groups of interbreeding populations that are reproductively isolated from other such groups [193]. A central principle of the BSC is reproductive isolation, which essentially means that the progeny of two different species (hybrids) are sterile, inviable or both, and consequently a genetic dead end. It has been of considerable interest to biologists to better understand how speciation occurs and how incipient species are reproductively isolated from one another. Attention has naturally turned towards genetic differences between closely related species, which might hold clues into how changes in biological processes lead to reproductive isolation between organisms.

Satellite DNA are some of the most rapidly evolving sequences in eukaryotic genomes [194]. Indeed, their tandem repetitive structure on chromosomes hinders accurate DNA replication and recombination, leading to a higher-than-normal mutation rate. Consistently, closely related species exhibit dramatic variations in their satellite DNA content, which can be observed as changes in copy number, changes in chromosomal location and changes in the underlying sequence [170,172,195]. The striking nature of these changes has led to many proposals that satellite DNA divergence plays a role in reproductive isolation between species [159,160,196–198].

Henikoff and colleagues proposed the centromere drive hypothesis, suggesting that centromeres with an altered satellite DNA content can affect progression through male and female meiosis [197,199]. While larger centromeres with increased satellite DNA copy number can bias their transmission into the oocyte during asymmetric female meiosis, centromeric imbalances can also result in meiotic defects and sterility during the symmetric male meiosis. As a result, centromeric histones co-evolve with the variant centromeres in order to maintain overall fitness of the species [197]. In this manner, centromeric satellite DNA repeats and associated proteins are engaged in persistent co-evolution. One unintended consequence of centromere drive is that isolated populations of the same species can experience different trajectories of centromere repeat/centromere protein co-evolution. Thus, these two populations may evolve incompatibilities in their chromosome segregation machinery leading to their reproductive isolation and speciation [197]. As such, chromosome segregation defects have been proposed to cause hybrid incompatibility [200–203] and hybrid incompatibility-like phenomena [204–207] in many species.

In contrast to centromeric satellite DNA, the consequences of pericentromeric satellite DNA divergence have been relatively poorly studied. The main reason is that a framework to understand pericentromeric satellite DNA function has been largely missing. Based on recent advances in our knowledge on pericentromeric satellite DNA and chromocenter function, we will highlight three notable cases where divergent pericentromeric satellite DNA sequences lead to deleterious outcomes in hybrids. All three cases are from *Drosophila* species where the divergent pericentromeric satellite DNA repeats between species are comprehensively mapped [172,208].

8.1. Hybrid embryonic lethality and the pericentromeric 359 bp satellite DNA

The first case focuses on crosses between *D. simulans* females and *D. melanogaster* males, species which diverged ~2–3 million years ago [209]. Here, the F_1 male hybrids are viable while the F_1 female hybrids are embryonic lethal [210]. Female embryonic lethality occurs around cycle 9/10 post fertilization, due to chromosome segregation defects of the *D. melanogaster* X chromosome (which is not present in male hybrids) [117]. Specifically, the ~11 Mb 359 bp satellite on the *D. melanogaster* X chromosome forms anaphase bridges during cell division, resulting in mitotic failure and embryonic lethality. Consistently, female hybrid embryonic lethality is rescued by the Zhr^1 allele, which contains a deletion of the 359 bp satellite DNA on the *D. melanogaster* X chromosome [211,212]. It is important to note that the 359 bp repeat is present in a smaller tract on the 3rd chromosome in addition to the ~11 Mb tract on the X chromosome [172]. Despite this, the 3rd chromosome 359 bp locus does not form anaphase bridges during female hybrid lethality [117]. Consistently, previous results have suggested that the size of the 359 bp tract on the *D. melanogaster* X chromosome does not cause lethality [213]. While the precise cause remains unidentified, female hybrid lethality is co-incident with zygotic genome activation, heterochromatin modifications at satellite DNA repeats and chromocenter assembly. Thus, it seems more likely that some aspect of gene expression, heterochromatin formation and/or chromocenter regulation is impaired specifically near the X chromosome 359 bp locus, leading to mitotic failure and embryonic lethality.

8.2. Hybrid male sterility and OdsH

The second case focuses on crosses between *D. simulans* and *D. mauritiana*, species which diverged ~250,000 years ago [209]. Here, F1 females are viable and fertile while F1 males are sterile. While many loci are thought to mediate hybrid sterility between these two species [214], introgression experiments isolated one such hybrid sterility factor, a rapidly evolving DNA-binding protein OdsH from *D. mauritiana* ($OdsH^{mau}$) [215]. More recent work suggests that $OdsH^{mau}$ elicits male sterility by aberrantly binding the satellite DNA-rich *D. simulans* Y chromosome [216]. This is in contrast to $OdsH^{sim}$, which typically only binds the *D. simulans* X chromosome and 4th chromosome. Moreover, the binding of $OdsH^{mau}$ on Y^{sim} resulted in a striking decondensation of the bound chromosome [216]. What remains incompletely understood is how $OdsH^{mau}$-dependent chromosome decondensation could lead to hybrid male sterility. One answer could be based on the important role for chromosome compaction during *Drosophila* male meiosis. Unlike *Drosophila* females where homologous chromosomes synapse prior to the first meiotic division, *Drosophila* male sequester their homologous chromosomes into chromosome territories before chromosome segregation during meiosis I [217]. The formation of chromosome territories that contain the two homologous chromosomes requires breakdown of the chromocenter, which typically clusters heterologous chromosomes. Interestingly, a new study has highlighted an important role for chromosome compaction by the condensin II complex during territory formation [218]. Moreover, condensin II mutants are male sterile and exhibit heterologous chromosome attachments during meiosis I, which leads to aneuploidy [219]. As such, defects in chromosome condensation in *D. simulans-D. mauritiana* male hybrids, mediated by mislocalization of $OdsH^{mau}$ may lead to ineffective chromocenter disruption during meiotic prophase I, aberrant chromosome associations and hybrid sterility.

8.3. Chromocenter disruption and micronuclei in sterile and inviable hybrids

The final case focuses on crosses between *D. melanogaster* females and males of the *D. simulans* complex (including *D. simulans* and *D. mauritiana*). Here, F_1 female hybrids are sterile but viable while F_1 male hybrids are lethal [210]. While the hybrid incompatibility genes between these species have been previously mapped, their functions have been largely addressed at the pure species level [210,220–223]. As a result, the underlying cellular defects in the hybrid that cause lethality and sterility have remained poorly understood. Based on reports that chromocenter formation was important for genome encapsulation, a recent investigation examined whether chromocenters were properly formed in hybrids containing divergent pericentromeric satellite DNA repeats [224]. Strikingly, cells in the germline and somatic tissues that are responsible for hybrid sterility and lethality displayed significant chromocenter disruption with species-specific pericentromeric satellite DNA repeats not clustering with each other. Moreover, the chromocenter disruption in hybrid cells was accompanied by micronuclei formation, consistent with chromocenter studies performed in pure species. Thus, both sterile and lethal hybrids between these species exhibit a conserved cellular phenotype of chromocenter disruption and micronuclei[224]. Notably, chromocenter disruption in the hybrid was dependent on the known hybrid incompatibility (HI) factors, Hmr^{mel} and Lhr^{sim}. Interestingly, these HI factors are DNA-binding proteins that are known to localize to centromeric and pericentromeric heterochromatin, as well as chromocenters in pure species [220,222,223,225]. While many functions have been proposed for these proteins, one intriguing study suggests that Hmr and Lhr may function to disengage sister chromatids during mitosis [220]. As a result, it has been proposed that Hmr/Lhr could aberrantly or precociously disassemble chromocenters in hybrid interphase cells, leading to micronuclei, cell death and hybrid incompatibility [224].

Although the function of chromocenters in genome encapsulation has only been tested in *Drosophila* and mouse, it should be noted that chromocenters are observed in a wide variety of eukaryotes suggesting that this function may be more widespread. Moreover, pericentromeric satellite DNA are highly divergent between closely related species across the eukaryotic domain [160] and could inhibit genome encapsulation in hybrids in a manner similar to what has been observed in *Drosophila* hybrids. Strikingly, studies have found that plant hybrids also fail to encapsulate their genomes, with species-specific chromosomes 'extruded' from the interphase nucleus [226,227]. Thus, these data suggest a simple paradigm for pericentromeric satellite DNA and chromocenter function within and across species. Within species, chromosomes containing identical repeats are clustered into an ensemble and encapsulated within a single nucleus (Fig. 2B). As a result, co-evolving pericentromeric satellite DNA repeats and cognate binding proteins function as a 'self' signal for the chromosomes of a species (Fig. 2B). In hybrids, divergent repeats and the resultant incompatibilities between chromocenter-associated proteins lead to impaired genome encapsulation and cell death. Although many facets of pericentromeric satellite DNA and chromocenter regulation remain to be discovered in pure species and hybrids, we would like to emphasize the importance of these repeats in overcoming an inherent logistical challenge of eukaryotic organisms: preserving the entire chromosome complement in a single nucleus.

9. Conclusions

Satellite DNA repeats are largely unmapped in most modern genome assemblies and thus still belong to the so-called 'dark genome'. In addition, their abundance and repetitive nature mean that conceptually straightforward loss-of-function experiments have been next-to impossible. As a result, the very functionality of these repeats has been often called into question, even though organisms dedicate a significant fraction of their biosynthetic capacity to these repeats and faithfully propagate them, generation after generation. In this review, we have discussed how the clustering of pericentromeric satellite DNA into chromocenters plays a role in important processes such as genome encapsulation, gene expression regulation and speciation. In this section, we would like to highlight two fascinating questions regarding satellite DNA that remain unaddressed. The first question relates to how satellite DNA copy number impacts its function. Specifically, we still do not understand how many repeats are required for a chromosome to cluster into chromocenters. However, we are encouraged by recent next-generation sequencing studies, which have quantified satellite DNA variation within and across species [169,195]. The recent telomere-to-telomere assemblies of the human genome are also a massive step forward in this regard [152]. Thus, we foresee that engineered changes in satellite DNA copy number may become tenable in the near future allowing us to address this question. The second question relates to the processes that facilitate the oft-observed dramatic variations in satellite DNA content between closely related species. While researchers have suggested replication- and recombination-based mechanisms to account for changes in microsatellite loci [194], changes in the substantially larger centromeric and pericentromeric satellite DNA remain poorly accounted for. We propose that understanding the natural processes that promote satellite DNA variation within species has important implications for reproductive isolation and the origin of new species.

Conflicts of interest

Franziska Brändle, Benjamin Frühbauer and Madhav Jagannathan have no conflicts of interest to declare.

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