CHAPTER TWO

Maternal inheritance of centromeres through the germline

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Abstract

The centromere directs chromosome segregation but is not itself genetically encoded. In most species, centromeres are epigenetically defined by the presence of a histone H3 variant CENP-A, independent of the underlying DNA sequence. Therefore, to maintain centromeres and ensure accurate chromosome segregation, CENP-A nucleosomes must be inherited across generations through the germline. In this chapter we discuss three aspects of maternal centromere inheritance. First, we propose mechanisms for maintaining CENP-A nucleosomes through the prolonged prophase arrest in mammalian oocytes. Second, we review mechanisms by which selfish centromeres bias their transmission through female meiosis. Third, we discuss regulation of centromere size through early embryonic development.

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1. Introduction

1.1 Epigenetic specification of centromeres

Accurate segregation of eukaryotic chromosomes is directed by a locus called the centromere. In most eukaryotes, with budding yeast as a notable exception, centromeres are not encoded by a particular DNA sequence, but are instead epigenetically specified by the presence of a histone H3 variant centromere protein A (CENP-A), independent of the underlying DNA sequence (Allshire & Karpen, 2008; Black & Cleveland, 2011). However, centromeres are typically associated with characteristic DNA sequences ranging from a 125 bp sequence necessary and sufficient for "point" centromeres in budding yeast (Bloom & Carbon, 1982; Fitzgerald-Hayes, Clarke, & Carbon, 1982; Panzeri & Philippsen, 1982; Saunders, Fitzgerald-Hayes, & Bloom, 1988) to highly diverged repetitive or a mixture of repetitive and non-repetitive sequences (Locke, Segraves, Carbone, et al., 2003; Piras, Nergadze, Magnani, et al., 2010; Shang, Hori, Toyoda, et al., 2010) in "regional" centromeres. Regional centromeres in humans, for example, contain up to 5 Mb of 171 bp alpha-satellite repeats (Waye & Willard, 1987).

Initial evidence for epigenetic specification of centromeres was the existence of naturally occurring neocentromeres, found on complex DNA sequences (i.e., not repetitive) (Barry, Howman, Cancilla, et al., 1999; Choo, 1997; Depinet, Zackowski, Earnshaw, et al., 1997; Scott & Sullivan, 2014), that can be inherited through multiple generations, indicating their function in mitosis and meiosis (Amor, Bentley, Ryan, et al., 2004; Tyler-Smith, Gimelli, Giglio, et al., 1999). Neocentromeres recruit CENP-A and other centromere proteins (Bassett, Wood, Salimian, et al., 2010; Stellfox, Bailey, & Foltz, 2013; Voullaire, Slater, Petrovic, & Choo, 1993), suggesting that typical repetitive centromere DNA sequences are unnecessary for centromere specification and that centromere propagation is epigenetic and conferred by the presence of CENP-A nucleosomes. In fact, targeting CENP-A or its dedicated histone chaperone to non-centromeric chromatin is sufficient to form functional centromeres and recruit other kinetochore proteins in *Drosophila* and human cells (Barnhart, Kuich, Stellfox, et al., 2011; Logsdon, Gambogi, Liskovykh, et al., 2019; Mendiburo, Padeken, Fulop, et al., 2011). Taken together, these studies support the centrality of CENP-A nucleosomes in specifying and maintaining the centromere locus through multiple cell cycles and across generations.

1.2 Self-propagating nature of CENP-A nucleosomes

CENP-A chromatin is maintained at the same locus on each chromosome from one cell cycle to the next to preserve centromere identity, preventing loss from a single chromosome or duplication to make a functional "dicentric" chromosome that leads to chromosome breakage at mitosis (McClintock, 1941). In fact, centromeric chromatin is quite immobile on the timescale of organismal generations. For example, two different higher order α-satellite arrays on human chromosome 17 act as epialleles (Maloney, Sullivan, Matheny, et al., 2012). Each one inherits the parental centromere location, which is then maintained independently on the maternal and paternal chromosomes. To maintain centromeric chromatin through each cell cycle, CENP-A is partitioned equally between sister chromatids during S-phase DNA replication, reducing levels to half at each centromere, and subsequently restored to full levels by deposition of new CENP-A by a dedicated chaperone (HJURP in mammals, Scm3 in yeast and CAL1 in flies) (Jansen, Black, Foltz, & Cleveland, 2007; Lagana, Dorn, De Rop, et al., 2010; Schuh, Lehner, & Heidmann, 2007) (Fig. 1). Assembly of new CENP-A nucleosomes near the existing CENP-A nucleosomes preserves the location and is achieved by specific interactions of CENP-A assembly factors with constitutive centromere proteins (e.g., CENP-C or CENP-A itself). This process is restricted to G1 as phosphorylation of CENP-A assembly factors (HJURP and Mis18BP1) by CDK1/2 inhibits assembly in late S (following CENP-A protein expression; Shelby, Vafa, & Sullivan, 1997), G2 and M (Dunleavy, Roche, Tagami, et al., 2009; Foltz, Jansen, Bailey, et al., 2009; Hayashi, Fujita, Iwasaki, et al., 2004; Silva, Bodor, Stellfox, et al., 2012) (Fig. 1).

Because existing CENP-A nucleosomes direct the deposition of new ones, centromeres are self-propagating in nature. In current models of centromere inheritance, interactions between the CENP-A nucleosome and its assembly factors are expected to be stoichiometric, so that recruitment of assembly factors would be proportional to the number of CENP-A nucleosomes. Thus, after equal partitioning between sister chromatids in S-phase, each CENP-A nucleosome would direct deposition of a new one, maintaining constant level over many cell cycles. This view of cell cycle-coupled assembly of CENP-A chromatin has emerged largely from experiments in tissue culture cells (Chen & Mellone, 2016; De Rop, Padeganeh, & Maddox, 2012; Erhardt, Mellone, Betts, et al., 2008;

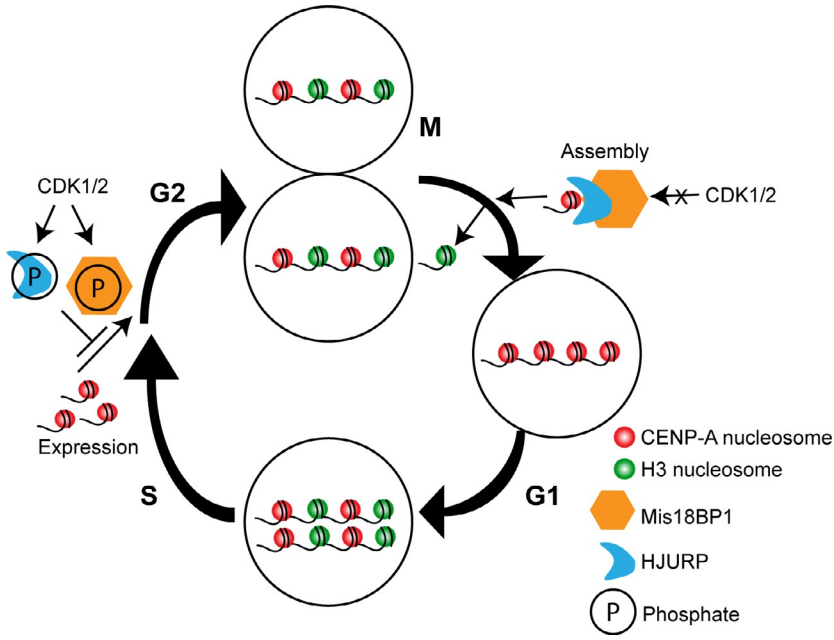

Fig. 1 Epigenetic inheritance cycle of CENP-A as established in cycling somatic cells. CENP-A levels are reduced to 50% in S and then restored by cell cycle-coupled chromatin assembly upon exit from mitosis, in G1. The CENP-A specific chaperone HJURP and its interacting partner Mis18BP1 are inhibited at S, G2 and M by CDK1/2 mediated phosphorylation.

Falk & Black, 2012). However, there are additional complexities to the transmission of CENP-A chromatin across generations via the germline. In this chapter, we discuss three key aspects of centromere inheritance from parent to progeny:

1. How is CENP-A chromatin maintained?
2. Biased inheritance of centromeres in female meiosis.
3. Regulation of centromere size in early development.

2. How is CENP-A chromatin maintained?

Centromere propagation through the germline requires the maintenance of CENP-A nucleosomes through gametogenesis, posing a challenge for the existing paradigm of centromere transmission in cycling somatic cells. Mammalian oocytes arrest in prophase I until meiotic resumption occurs, which could be up to 2 years in mice or decades in humans.

Therefore, the mammalian maternal germline faces the challenge of maintaining CENP-A chromatin through this prolonged prophase arrest. A parallel challenge exists in the male germline as CENP-A nucleosomes must be preferentially retained when other bulk histones are exchanged for protamines in a dramatic genome-wide reorganization (Bao & Bedford, 2016; Gaucher, Reynoird, Montellier, et al., 2010; Rathke, Baarends, Awe, & Renkawitz-Pohl, 2014). Here we propose two possible mechanisms to maintain CENP-A nucleosomes through the extended prophase arrest in oocytes: (1) stability of the chromatin-assembled CENP-A nucleosomes with little or no decay and/or (2) continual replenishment of CENP-A nucleosomes by new CENP-A assembly to compensate for decay during the prophase arrest.

2.1 CENP-A stability

Evidence from mammalian cycling somatic cells demonstrates that CENP-A nucleosomes are remarkably stable and persist at centromeres over several cell cycles with no detectable turnover (Bodor, Mata, Sergeev, et al., 2014; Bodor, Valente, Mata, et al., 2013). Furthermore, experiments with tagged CENP-A in plants and flies corroborate the idea that G1 assembled CENP-A chromatin can stably propagate centromere identity from one somatic cell cycle to the next (Lermontova, Koroleva, Rutten, et al., 2011; Schuh, Lehner, & Heidmann, 2007). In the mouse germline, an oocyte-specific conditional knockout of CENP-A early in the prophase I arrest does not affect fertility or CENP-A levels at oocyte centromeres. This result indicates that CENP-A is retained at centromeres during the prophase I arrest with no detectable assembly of newly expressed CENP-A during the reproductive lifespan of the animal, and that centromeric chromatin assembled prior to meiotic entry is sufficient for centromere function and inheritance (Smoak, Stein, Schultz, et al., 2016). Taken together, these studies highlight the remarkable stability of CENP-A nucleosomes at the centromere, but the factors that contribute to this stability are unclear.

One candidate for providing CENP-A stability is its intrinsic structural rigidity. Biophysical and structural studies have revealed that the internal dynamics of the $(CENP\text{-}A/H4)_2$ heterotetramer are different compared to its counterpart histone complex, the $(H3/H4)_2$ heterotetramer (Black, Foltz, Chakravarthy, et al., 2004; Sekulic, Bassett, Rogers, & Black, 2010). So-called "hydrophobic stitches" generated by substitutions of six amino acids in CENP-A relative to its counterpart, histone H3, provide

conformational rigidity to the interface of CENP-A with its histone partner, H4 (Sekulic et al., 2010). Replacement of these six amino acids with the counterpart H3 residues reduces the steady-state level of accumulation at centromeres while leaving interactions with the CENP-A chaperone, HJURP, intact (Bassett, DeNizio, Barnhart-Dailey, et al., 2012). Could this conformational rigidity help retain CENP-A at centromeres during the prolonged prophase arrest in mammalian oocytes? Some clues are available from experiments in other systems. For instance, mutating one of the hydrophobic stitch residues in plants results in severely impaired localization of centromeric CENP-A (CENH3), consistent with a role of the hydrophobic interface in stabilizing CENP-A nucleosomes (Karimi-Ashtiyani, Ishii, Niessen, et al., 2015). Another H3 variant, the testis specific histone H3.5, forms an unstable nucleosome that has been attributed to the presence of a Leu residue at the interface with histone H4 (position 103 in human) instead of the bulkier hydrophobic phenylalanine residue present in canonical H3 or other H3 variants (Schenk, Jenke, Zilbauer, et al., 2011; Tachiwana, Kagawa, Osakabe, et al., 2010; Urahama, Harada, Maehara, et al., 2016).

Non-histone centromere proteins, such as the 16 constitutive centromere associated network (CCAN) proteins, could also contribute to CENP-A stability (Cheeseman & Desai, 2008; Hori, Shang, Takeuchi, & Fukagawa, 2012; Perpelescu & Fukagawa, 2011). Two of these proteins, CENP-C and CENP-N, are direct binding partners of CENP-A (Carroll, Silva, Godek, et al., 2009; Guse, Carroll, Moree, et al., 2011; Kato, Jiang, Zhou, et al., 2013) and contribute to CENP-A retention at centromeres. CENP-C can reshape CENP-A nucleosomes, generating stability at multiple locations within the folded histone core, and is important for retaining the pool of assembled CENP-A nucleosomes in mitotic cell cycles (Falk, Guo, Sekulic, et al., 2015; Falk, Lee, Sekulic, et al., 2016). In addition, flies with impaired CENP-C function have reduced CENP-A at centromeres in spermatids, suggesting a potential role in centromere maintenance during early meiosis (Kwenda, Collins, Dattoli, & Dunleavy, 2016). CENP-N contacts both CENP-A and nucleosomal DNA, cross-bridging the molecules in a manner that likely imparts stability to CENP-A nucleosomes in mitotically-cycling cells (Guo, Allu, Zandarashvili, et al., 2017; Pentakota, Zhou, Smith, et al., 2017). The role of either CENP-C or CENP-N is yet to be determined in oocytes.

2.2 Continual replenishment of CENP-A in germline

An alternative to a structural maintenance mechanism is the possibility that CENP-A nucleosomes are replenished by new assembly during the meiotic prophase arrest to compensate for potential CENP-A decay, thereby maintaining CENP-A chromatin. CENP-A can gradually assemble at a slow rate of 2% per day in prophase-arrested starfish oocytes cultured *in vitro*. Furthermore, when the essential assembly component Mis18 binding protein 1 (Mis18BP1) was depleted, these oocytes experienced segregation errors, suggesting that CENP-A nucleosomes are normally replenished gradually over time (Swartz, McKay, Su, et al., 2018). This finding is consistent with studies in *Drosophila* and plants that report gradual CENP-A nucleosome assembly in prophase I in the male germline (Dunleavy, Beier, Gorgescu, et al., 2012; Raychaudhuri, Dubruille, Orsi, et al., 2012; Schubert, Lermontova, & Schubert, 2014). However, these studies appear inconsistent with the apparently stable CENP-A nucleosomes observed in prophase-arrested mouse oocytes. CENP-A injected into oocytes cultured *in vitro* on short timescales (up to 2 days) does not localize to centromeres (Smoak et al., 2016), arguing against new CENP-A nucleosome assembly during the prophase arrest. In addition, aged mice have ~30% lower levels of CENP-A nucleosomes than young mice (Smoak et al., 2016), inconsistent with regulation through a homeostasis mechanism including nascent deposition. This reduction over time is not affected by deletion of the CENP-A gene early in the prophase arrest, ruling out deposition of newly expressed CENP-A protein during the extended arrest. One possibility to reconcile these conflicting results is that a stable pool of CENP-A protein slowly cycles on and off centromeres during the prophase arrest, which would not require new transcription. Thus, further studies are necessary to test a requirement for the CENP-A assembly machinery in prophase-arrested mammalian oocytes.

In contrast to the organisms discussed above, some do not require CENP-A maintenance in the female germline. For instance, worms remove CENP-A nucleosomes in the female germline and re-assemble them *de novo* during embryogenesis (Monen, Maddox, Hyndman, et al., 2005). Similarly, CENP-A is undetectable on *Arabidopsis* egg chromatin and is assembled *de novo* in the zygote (Ingouff, Rademacher, Holec, et al., 2010). The loss of CENP-A in the female germline in worms and plants is certainly in contrast to the robust stability of CENP-A chromatin in mammalian oocytes

(Smoak et al., 2016), suggesting that plants and worms have evolved an active CENP-A removal pathway in the germline that mammals—and presumably many other eukaryotes—lack.

3. Biased inheritance of centromeres through female meiosis: Centromere drive

Female meiosis is asymmetric, in that only one of the four possible division products become the egg while the others are degraded, providing a clear opportunity for selfish genetic elements to cheat, as any chromosome that can preferentially segregate to the egg has a transmission advantage (Chmátal, Schultz, Black, & Lampson, 2017; Pardo-Manuel de Villena & Sapienza, 2001). The first example of preferential transmission was observed in maize (Rhoades, 1942), where motor proteins recruited to repetitive selfish DNA elements (Dawe, Lowry, Gent, et al., 2018) drive preferential meiotic segregation. The term "meiotic drive" was later introduced to emphasize the key role of asymmetric female meiosis in transmission ratio distortion (Sandler & Novitski, 1957). The centromere, which mediates attachment to the spindle and directs segregation, has the potential to cheat in female meiosis and bias its own inheritance. Drive by diverged repetitive centromeric satellite DNA and the antagonistic coevolution of centromere proteins was proposed to explain the "centromere paradox": the unexpected rapid evolution of centromere DNA and essential centromere proteins with conserved functions (Henikoff, Ahmad, & Malik, 2001). According to the centromere drive hypothesis, selfish centromere DNA sequences that increase their transmission through female meiosis also incur fitness costs, possibly due to linked deleterious alleles or mis-segregation in male meiosis. Consistent with this hypothesis, expansion of repetitive centromere DNA is associated with a driving locus in monkeyflowers that exhibits a strong transmission bias in female meiosis and significantly reduced pollen viability in males (Fishman & Saunders, 2008). These costs would provide selective pressure promoting rapid evolution of centromere proteins to suppress drive.

Another example of centromere drive is preferential segregation of Robertsonian (Rb) fusions, common chromosomal rearrangements formed by two telocentric chromosomes (centromere at the end) joining at their centromeres to create one metacentric chromosome (internal centromere) (White, Bordewich, & Searle, 2010). Preferential transmission of Rb fusions correlates with the size of the fusion centromere relative to

centromeres of the homologous unfused telocentrics, defined by levels of centromere proteins (Chmátal, Gabriel, Mitsainas, et al., 2014). In oocytes heterozygous for a single Rb fusion, both CENP-A and the major microtubule-binding protein NDC80 kinetochore complex component (NDC80)/highly expressed in cancer (HEC1) are enriched on the telocentric chromosomes that preferentially remain in the egg, relative to the homologous metacentric fusion that preferentially segregates to the polar body. Moreover, in a natural metacentric population that accumulated Rb fusions (CHPO strain), the fusion centromeres are enriched for these same proteins relative to the telocentric chromosomes. Further support for the drive hypothesis comes from holocentric plants (in which the centromere is present all over the chromosome) like *Luzula* where centromeres are not rapidly evolving since no single DNA element can bias its segregation, and species with symmetric meiosis that do not show signs of adaptive centromere evolution (Zedek & Bureš, 2016a, 2016b).

Conceptually, selfish segregation of centromeres in female meiosis depends on functional differences between centromeres on homologous chromosomes and asymmetric interactions with the metaphase I spindle that favors preferential orientation of the driving centromere toward the egg. These mechanisms have been studied in mouse systems with large differences in centromere DNA. For example, in a cross between two mouse strains (CF-1 and CHPO) with widely different amounts of centromeric minor satellite repeats, the larger centromeres have ~10-fold larger arrays of satellites, build larger kinetochores, and preferentially orient toward the egg side of the spindle. The small CHPO satellite arrays limit CENP-A nucleosome assembly to maintain small centromeres relative to the CF-1 centromeres even when they share the same nucleoplasm (Iwata-Otsubo, Dawicki-McKenna, Akera, et al., 2017). These larger centromeres exploit an asymmetry in α-tubulin tyrosination within the meiosis I spindle. Spindle asymmetry arises after migration to the cortex and depends on cortical polarization by the RAN GTPase and activation of the membrane-associated CDC42 GTPase (Akera, Chmátal, Trimm, et al., 2017).

To exploit spindle asymmetry, the larger centromeres preferentially detach from the cortical side and re-orient (or flip) toward the egg side. The larger centromeres initiate flipping at a higher frequency than the smaller centromeres and recruit more microtubule destabilizing activities, such as the depolymerizing kinesin-13, MCAK (mitotic centromere associated kinesin). MCAK favors tyrosinated microtubules, potentially explaining preferential flipping from the cortical side and more stable

orientation toward the egg side (Akera et al., 2017; Akera, Trimm, & Lampson, 2019) (Fig. 2A). This strategy of enriching destabilizing activity to win in female meiosis was also found in an interspecific cross between *Mus musculus* and *Mus spretus*. Although *spretus* centromeres have substantially more centromere DNA repeats, the recruitment of centromere proteins to *musculus* and *spretus* centromeres was similar in the hybrid. The repeats are not limiting in either case, as CENP-A nucleosomes number in the hundreds out of several thousand centromeric nucleosomes in mouse (Bodor et al., 2014; Iwata-Otsubo et al., 2017) (except for CHPO). Instead, the *spretus* centromeres recruit more MCAK by a mechanism based on differences in Condensin localization to gain a transmission advantage over the *musculus* centromeres (Akera et al., 2019). Thus, although winning centromeres in both the intraspecific and interspecific hybrid mouse models

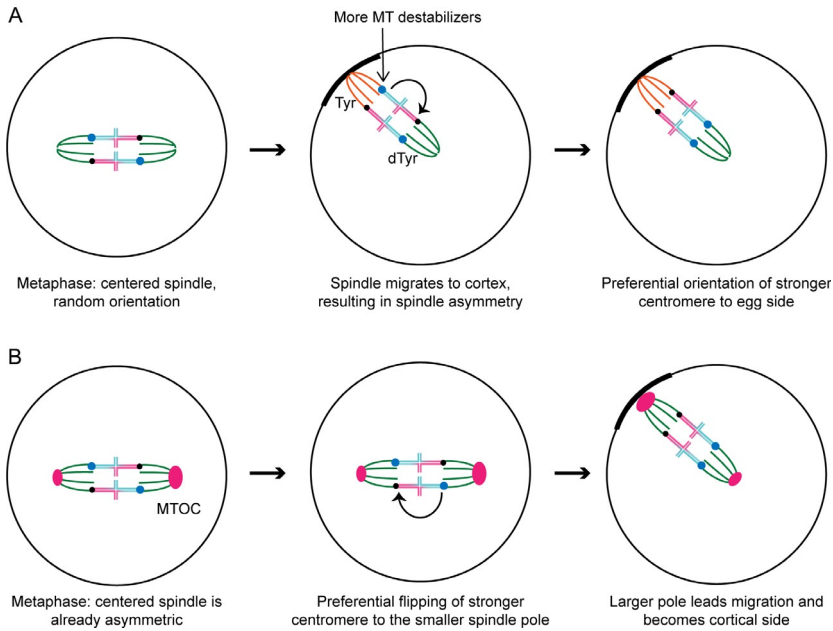

Fig. 2 Divergent mechanisms of centromere drive in female meiosis. (A) In the intraspecific CF1/CHPO hybrid oocyte, the spindle is asymmetric in tubulin tyrosination after cortical migration, and enrichment of microtubule destabilizers at stronger centromeres (blue) drives preferential flipping to the egg pole. (B) In the intraspecific BL6/SJL hybrid oocyte, the spindle poles (pink) are asymmetric prior to migration. In this case, stronger centromeres flip preferentially toward the smaller spindle pole and are retained in the egg as the larger pole leads migration toward the cortex.

exploit microtubule destabilizing activity to promote preferential orientation toward the egg pole in meiosis I, the mechanism for enriching destabilizers is distinct.

Another example of a diverged mechanism to achieve preferential inheritance was found in a different intraspecific cross (BL6 × SJL) with two chromosomes with driving centromeres (Fig. 2B). In this case the spindle was asymmetric with differences in tubulin and MTOC (microtubule organizing center) density between the two sides, prior to spindle migration to the cortex (Wu, Lane, Morgan, & Jones, 2018). The winning centromeres preferentially orient toward the pole with greater density of MTOCs, which is destined to become the egg side. These findings imply a different mechanism to establish spindle asymmetry, independent of cortical signals, as well as directional spindle migration to orient the larger spindle pole toward the cortex.

The centromere drive hypothesis predicts that systems with drive would also evolve suppression mechanisms (Henikoff et al., 2001). For example, any mechanism that reduces flipping events has the potential to negate drive if it prevents selfish centromeres from orienting toward the egg side of the spindle. This could be achieved by reducing the time available for flipping, which is borne out by the observation that the *spretus/musculus* hybrid does not show a bias in segregation unless anaphase is artificially delayed (Akera et al., 2019). As another potential suppression mechanism, centromere binding proteins could evolve to modulate binding affinity to expanded repetitive DNA and equalize centromeres. For example, if maternal and paternal centromeres differ in the zygote after fertilization, this asymmetry could be attenuated during early embryonic cell cycles to equalize centromeres. Therefore, evolution of centromere proteins to suppress centromere drive may be a key factor in determining centromere size, with a molecular "tug-of-war" between forces that generate expanded centromeres that can drive and those that limit centromere size while maintaining enough CENP-A chromatin to support centromere function.

4. Regulating centromere size in early development

4.1 Centromere-mediated genome elimination: Importance of equivalent centromeres

The importance of close equivalency in centromere size and function across chromosomes is exemplified in plant crosses with uniparental genome

elimination, generating haploid plants (Comai & Tan, 2019; Ishii, Karimi-Ashtiyani, & Houben, 2016; Riera-Lizarazu, Rines, & Phillips, 1996; Sanei, Pickering, Kumke, et al., 2011). If parents are from different species, or one parent expresses a mutant CENP-A, centromere asymmetry in the zygote can result in elimination of the genome with the smaller/mutant centromere in early embryonic development (Ravi & Chan, 2010). This is not only an interesting phenomenon for understanding the role of centromeric chromatin in genome maintenance, but also useful as a rapid method to generate haploid plants to accelerate genetic manipulations (i.e., for agricultural biotech efforts) (Ishii et al., 2016; Ravi, Marimuthu, Tan, et al., 2014). The key factor driving centromere-mediated genome elimination appears to be an inability to resolve large differences in either size or function (i.e., wild type or mutant CENP-A) between the parental centromeres, whereby the larger/wild type centromere outcompetes the smaller/mutant counterpart (Fig. 3) (Wang & Dawe, 2018). During genome elimination, the presumed inability of the smaller/mutant centromere to recruit and assemble new CENP-A results in perturbed interactions with spindle microtubules and subsequent mis-segregation in the zygote (Sanei et al., 2011).

Several lines of evidence illustrate that large differences between parental centromeres can result in centromere-mediated genome elimination (Ishii et al., 2016; Wang & Dawe, 2018). For example, crossing an *Arabidopsis*

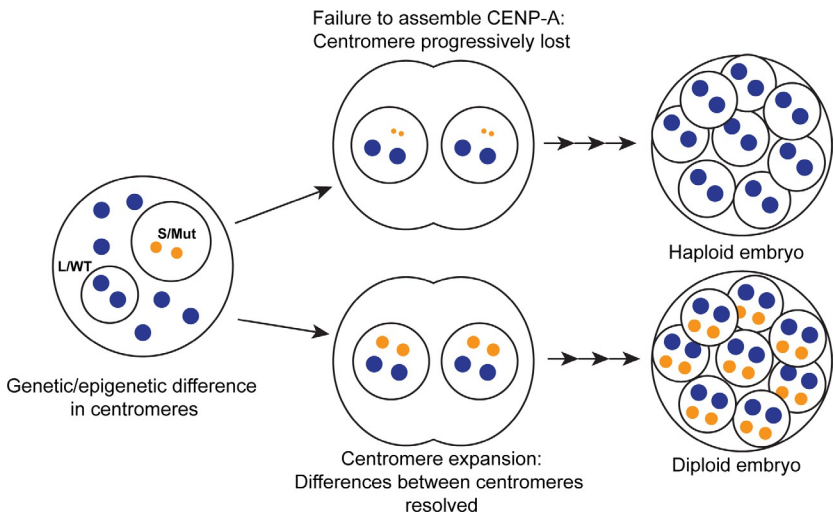

Fig. 3 Centromere-mediated genome elimination in embryos. Centromere dimorphism between parents can either be due to a size difference between two species (larger/L vs smaller/S) or due to one parent carrying a mutant CENP-A (WT/Mut). The smaller/mutant centromeres are often eliminated unless they expand during early development.

plant with mutant CENP-A to a wild type plant generates haploids containing only the genome with wild type CENP-A (Ravi & Chan, 2010). In this case the centromeres with mutant CENP-A nucleosomes lose to those with WT CENP-A in terms of nascent nucleosome assembly in each cell cycle or stability once assembled (Ravi, Shibata, Ramahi, et al., 2011). The embryos from this cross have high aneuploidy rates, along with haploidy, highlighting the fact that centromere size asymmetry in early development can lead to segregation errors. Point mutations in the histone fold domain of CENP-A (L130F in Arabidopsis or L106F in sugar beet, corresponding to the known hydrophobic stitch residue [see above] L91 in humans), which greatly reduce centromere localization of the mutant CENP-A nucleosomes in gametes, can also induce haploids but at a lower frequency (Karimi-Ashtiyani et al., 2015). Another classic case of genome elimination is a cross between *H. vulgare* and *H. bulbosum*, where despite having comparable centromere sizes in the two parents, haploids are still generated at a high rate with the *H. bulbosum* genome eliminated due to a parent-specific loss of CENP-A on the *H. bulbosum* chromosomes (Sanei et al., 2011). This is a unique example of genome elimination occurring due to differing properties but not size of CENP-A chromatin, such that one is incompatible for templating assembly of the other, leading to progressive loss of CENP-A on the *H. bulbosum* chromosomes.

What determines whether the genome with smaller/mutant centromeres will be eliminated? The balance between factors that influence either degradation of the smaller/mutant centromere or expansion through *de novo* CENP-A assembly may determine whether that genome will be lost or survive in the cross (Wang & Dawe, 2018). For example, a cross between oat (larger centromere species) and maize (smaller centromere species) usually results in haploid oat plants with complete elimination of the maize genome early in development (Kynast, Riera-Lizarazu, Vales, et al., 2001; Riera-Lizarazu et al., 1996), except in rare cases where the maize genome can survive at low frequencies in oat when the maize centromeres expand, thus reducing the size disparity (Wang, Wu, Zhang, et al., 2014). Taken together, these studies support the idea that differences between parental centromeres in the embryo arising from disparate centromere size, mutations in CENP-A nucleosomes or incompatibility between CENP-A from different species are detrimental to development of the organism. By extension, mechanisms that reduce centromere size dimorphism are likely crucial to avoid genome elimination or aneuploidy. Such mechanisms would also suppress centromere drive by equalizing maternal and paternal centromeres in the embryo.

4.2 Resolving centromere differences in early embryos

In theory, centromeres of disparate size might equalize by either a redistribution of the chromatin bound fraction to achieve comparable centromere sizes on all chromosomes or specific expansion of the smaller centromere by preferential assembly. Related to this point, following reduction in CENP-A levels by RNAi-mediated knockdown in *Drosophila* sperm, progeny maintain reduced levels of CENP-A chromatin even when CENP-A expression is restored (Raychaudhuri et al., 2012). This result is consistent with the idea that the abundance of CENP-A nucleosomes provides the epigenetic information to set the size of the centromere in the next generation. However, even though CENP-A is knocked down only in the sperm and not the egg, CENP-A levels in the progeny are reduced on both maternal and paternal centromeres of the autosomes, suggesting a mechanism to equalize CENP-A levels. Perhaps the fly example is a less extreme case of the instances in other species (*C. elegans* and *A. thaliana*, mentioned above) where CENP-A is lost during reproduction and subsequently "reset" during embryogenesis (Ingouff et al., 2010; Monen et al., 2005). Conversely, in centromere drive systems, asymmetry between centromeres of homologous chromosomes persists, ultimately leading to biased segregation in female meiosis. In such cases, for example the mouse hybrids discussed above, differences in repetitive centromere DNA may contribute to the persistent asymmetry (Akera et al., 2019; Iwata-Otsubo et al., 2017). Overall, these studies raise questions (see below) related to CENP-A chromatin inheritance and mechanisms to eliminate centromere asymmetry. In particular, the balance of genetic (centromere DNA sequence) and epigenetic (differences in CENP-A) contributions to centromere inheritance remain unclear.

5. Conclusions and future perspectives

Overall, although we know that centromere location is defined by an epigenetic mark that directs its own propagation through cell divisions (and through organismal generations in many eukaryotic species), the exact mechanisms by which centromeres persist in the germline are still unclear. Some outstanding issues for the field to address in the coming years are:

(1) What are the molecular factors that contribute to CENP-A nucleosome stability through the extended prophase arrest in the mammalian female germline, and how does nascent CENP-A assembly contribute to maintaining this epigenetic mark?

(2) How do centromeres bias their segregation in female meiosis? Mouse models reveal the importance of asymmetry both in microtubule destabilizing activity between centromeres of homologous chromosomes and between the two sides of the meiotic spindle. How winning centromeres preferentially destabilize interactions with the cortical side of the spindle is unclear. Furthermore, little is known about fitness costs associated with driving centromeres or mechanisms that may have evolved to minimize these costs. New cell biological and genetic model systems may provide insights into these questions.

(3) What are the mechanisms regulating centromere size in the embryo? Studies of uniparental genome elimination suggest that centromeres either reach comparable size or smaller centromeres are inactivated by loss of CENP-A chromatin (Wang & Dawe, 2018). Direct evidence for equalization in the embryo is limited, however, and exploring CENP-A assembly on maternal and paternal chromosomes in early embryos promises to provide insight into how CENP-A chromatin is regulated on the two parental genomes. A putative equalization mechanism must differ from the model of centromere propagation purely by stoichiometric interactions between CENP-A and its assembly factors, and may therefore reveal a new paradigm for centromere inheritance. Finally, an important model to test is whether the mechanisms that reduce centromere strength imbalances in embryos evolved to suppress centromere drive.

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