Biochemical Society Transactions (2022) **50** 675–688
https://doi.org/10.1042/BST20200267

Review Article

Mitotic drive in asymmetric epigenetic inheritance

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Asymmetric cell division (ACD) produces two daughter cells with distinct cell fates. This division mode is widely used during development and by adult stem cells during tissue homeostasis and regeneration, which can be regulated by both extrinsic cues such as signaling molecules and intrinsic factors such as epigenetic information. While the DNA replication process ensures that the sequences of sister chromatids are identical, how epigenetic information is re-distributed during ACD has remained largely unclear in multicellular organisms. Studies of *Drosophila* male germline stem cells (GSCs) have revealed that sister chromatids incorporate pre-existing and newly synthesized histones differentially and segregate asymmetrically during ACD. To understand the underlying molecular mechanisms of this phenomenon, two key questions must be answered: first, how and when asymmetric histone information is established; and second, how epigenetically distinct sister chromatids are distinguished and segregated. Here, we discuss recent advances which help our understanding of this interesting and important cell division mode.

Introduction

A multicellular organism is composed of many distinct cell types which can originate from asymmetrical cell divisions (ACDs) [1–6]. Cells derived from ACD inherit identical genetic information but display different cellular properties and perform diverse functions. These distinct features could be regulated by the differential gene expression in different cell types, which can be extensively regulated by epigenetic mechanisms. Perturbation of proper epigenetic regulation may cause mis-determination of cell fates or cell fate maintenance failure, which can cause cancers, tissue dystrophy, infertility, and ageing [7,8].

Histone proteins are a major carriers of epigenetic information that help specify cell identities [9,10]. Previous studies in *Drosophila* male germline stem cells (GSCs) have shown that pre-existing (old) *versus* newly synthesized (new) histones H3 and H4 are asymmetrically inherited by the two daughter cells [11–14]. These results demonstrate that the sister chromatids carry distinct populations of histone proteins and segregate non-randomly during ACD in male GSCs. Furthermore, by introducing a dominant negative unphosphorylable point mutation, H3 threonine 3 to alanine (H3T3A), to disrupt asymmetric H3 segregation in GSCs leads to randomized H3 segregation in *Drosophila* male GSCs [14]. This can result in a spectrum of cellular defects, such as early-stage germ cell tumor, loss of GSCs and male fertility over time. These results suggest that asymmetric histone inheritance is critical for both stem cell maintenance and cellular differentiation. Alternatively, it is also plausible that the spectrum of defects is due to cellular defects arising due to mutation of histone marks required for defining distinct cell identities instead of required for the inheritance. However, how microtubules recognize and attach to sister centromeres in a selective manner to ensure non-random segregation of sister chromatids remains elusive. Centromeres are specific chromosomal regions that are epigenetically defined by CENtromere Protein A (CENP-A), a histone H3 variants [15–19]. Interestingly, CENP-A has been shown to be inherited asymmetrically in *Drosophila* male GSCs [20]. Additionally, in *Drosophila* Intestinal Stem Cells (ISCs), old CENP-A is inherited preferentially by the self-renewed ISCs [21]. These studies demonstrate that CENP-A segregates asymmetrically in adult stem cells, similar to the canonical histones H3 and H4. Furthermore, previous studies have revealed that 'mother' and 'daughter' centrosomes in stem cells are inherited nonrandomly as seen in male and

Received: 11 August 2021
Revised: 29 March 2022
Accepted: 30 March 2022

Version of Record published:
19 April 2022

Biochemical Society Transactions (2022) 50 675–688
https://doi.org/10.1042/BST20200267

female GSCs, neuro-progenitor cells in flies and radial glia progenitors in mice [22–25]. However, whether and how asymmetric sister centromere and non-random centrosome inheritance events coordinate in asymmetrically dividing cells to regulate asymmetric epigenetic inheritance need to be further investigated.

On the other hand, DNA strands can also undergo epigenetic modifications, such as DNA methylation. Previously, the inheritance patterns of DNA strands were investigated in *Drosophila* male GSCs using the CO-FISH (chromosome orientation fluorescence *in situ* hybridization) technique [26]. By using strand-specific probes to distinguish Watson *versus* Crick stands in CO-FISH, the sex chromosomes (i.e. X and Y chromosomes) displayed a nonrandom inheritance pattern, with GSCs inheriting the same DNA strands ~85% of the time. Whereas, the two major autosomes (i.e. second and third chromosomes) showed a random segregation pattern at nearly 50: 50 chances with each DNA strand [27]. Intriguingly, autosomes demonstrated co-segregation patterns with either two Watson or two Crick DNA strands, but not one of each to be inherited by the self-renewed GSC. However, the mechanism underlying this phenomenon remains elusive. Together, these results indicate the possibility of bias in DNA strand inheritance, either by nonrandom segregation or by co-segregation in a chromosome-specific manner. While these results oppose the 'immortal strand' hypothesis, such biased DNA strand inheritance may serve to transmit distinct epigenetic information carried by the two sister chromatids into the daughter cells during ACD, such as H3, H4, or CENP-A [1,27–29].

In summary, these discoveries in *Drosophila* male GSCs [1,3,4] using this excellent model to study ACD [8,30–32] have established a paradigmatic system to study the molecular and cellular mechanisms of asymmetric epigenetic inheritance. Gaining insights into the spatiotemporal regulation of these phenomena in male GSCs will improve our understanding of how asymmetric histone inheritance is related to distinct cell fate decisions in multicellular organisms.

Establishment of asymmetric epigenome

Histones: a carrier of epigenetic marks

In eukaryotes, canonical histone proteins form an octamer, which contains two copies of each core histone H3, H4, H2A, and H2B. The DNA wraps around these histone octamers to form nucleosomes which facilitate the packaging of DNA molecules [33–38]. In addition, histone proteins carry numerous post-translational modifications (PTMs), such as methylation, acetylation, ubiquitination and phosphorylation, etc. [9,39–42]. These modifications are involved in multiple cellular functions, including cell cycle progression, DNA damage repair, transcriptional regulation and cellular identity [43–50]. At each cell cycle, cells must re-distribute PTMs from old histones, which exist prior to S-phase and carry PTMs from the previous cell cycle, and new histones, which are synthesized during S-phase without PTMs from the previous cell cycle and will either 'copy' old histone PTMs or carry new ones [51–58].

Using mass spectrometry and stable isotope labeling of amino acids, a differential distribution of multiple histone PTMs on old *versus* new histones have been revealed in several human cell lines, which can be grouped into three categories based on their distributions during mitosis [59,60]. First, acetylation and lysine monomethylation are either symmetrically distributed between old and new histones or enriched on new histones. Second, di- and tri-methylation are mostly enriched on old histones. Third, phosphorylation of histones on H3S10, H3T3, H3T6, H3.1/2S28, and H1.4S26 residues are all enriched on old H3 at early mitosis [59]. Furthermore, it has been demonstrated that specific PTMs, such as H3K27me3 and H4K9me3, require more than one cell cycle to be re-established, suggesting that the new histones may require more time to resume those PTMs carried by the old histones in cultured cells [58].

In addition, it has been shown that certain enriched PTMs could serve as pluripotency markers in human embryonic stem cells (hESCs), such as hyperacetylation at H3K4, K9, K14, K18, K56 and K122 [60]. Conversely, the methylation of histone H3 at K9, K20, K27 and K36 have been suggested for their roles in initiating cellular differentiation [60]. Studies in *Drosophila* male GSCs have further revealed the potential roles of the differential phosphorylation of H3T3 and H3S10 during early mitosis, which are critical for stem cell maintenance [14,61]. Together, these findings suggest that the histone PTMs could indicate and regulate the differentiation status of stem cells.

DNA replication: an opportunity to establish asymmetric epigenome

Canonical histones are mainly transcribed and translated endogenously during S phase [62,63]. In principle, both old histones and new histones are present at the replication fork. Even though the mechanisms behind

new histone incorporation during DNA replication have been widely studied [64,65], less had been understood regarding how old histones are recycled until recently [57,66–68]

Here we will discuss recent findings with a focus on the patterns and mechanisms underlying old histone recycling in different systems. Recently, a series of genomics studies have shed light on such patterns and mechanisms in symmetrically dividing cells, such as mouse embryonic stem cells (mESCs) and budding yeast. Using the SCAR-seq (sister chromatids after replication by DNA sequencing) technique in mESCs, the old histone deposition pattern is tracked with *de novo* DNA replication at a genome wide level. Results from this have revealed that old histones H3 and H4 are recycled to both leading and lagging DNA strands, with a slight bias towards the leading strand (Figure 1B) [66]. A recent study using a similar method in human retinal pigment epithelial (hRPE-1) cells indicates this bias could be even stronger [69]. MCM2 is an essential subunit of the CMG (Cdc45–MCM–GINS) DNA helicase and can also act as a chaperone in recycling old histones toward the lagging strand. Mutations at the histone binding sites of the MCM2 have significantly increased the amount of old histone recycling towards the leading strand [66]. Using the eSPAN (Enrichment and Sequencing of Protein-associated Nascent DNA) technique, similar studies have been performed in budding yeast. These results have further substantiated that replication proteins could function as histone chaperones in regulating the Replication-Coupled Nucleosome Assembly (RCNA) process at the replication fork. For example, two subunits of the leading-strand DNA polymerase ε (Pol ε), DPB3 and DPB4, facilitate old histone deposition onto the leading strand (Figure 1C) [68]. Loss-of-function of either subunit leads to the incorporation of old histones biased toward the lagging strand. Furthermore, it has been demonstrated that PCNA

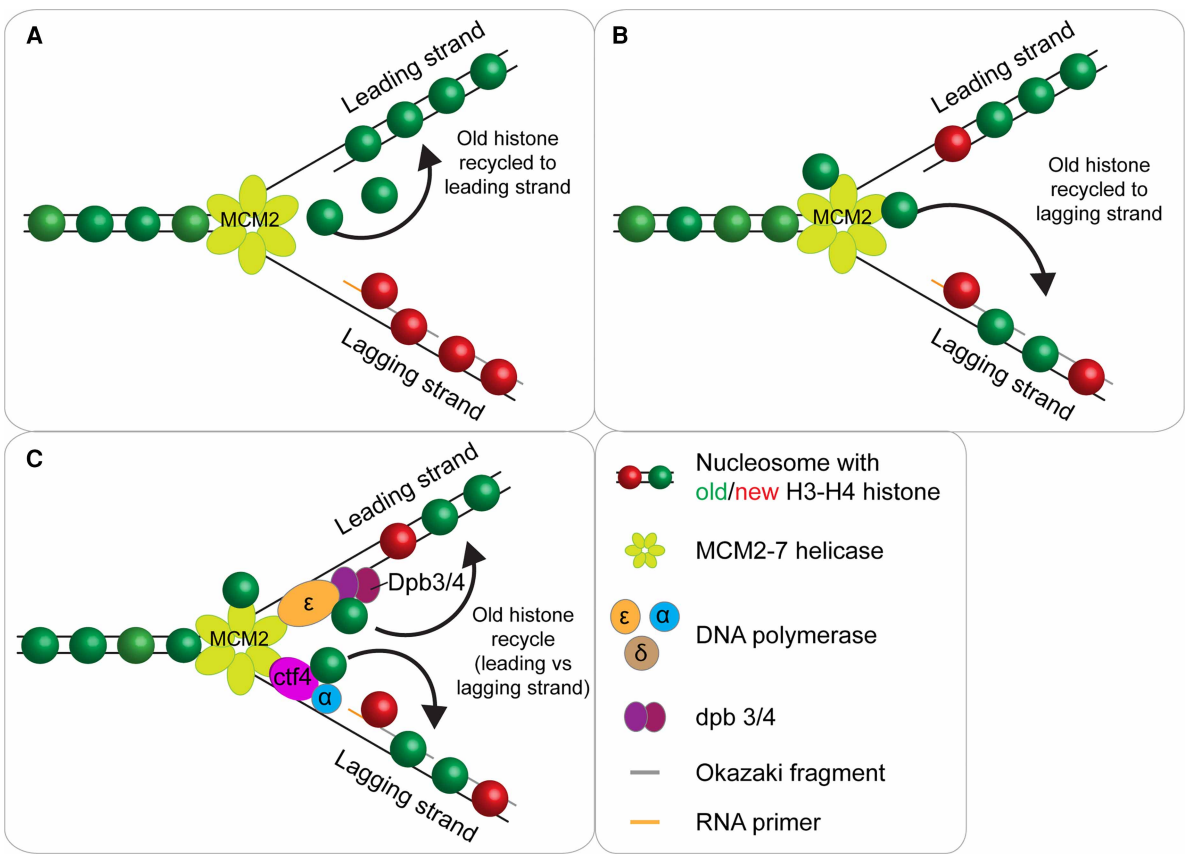

Figure 1. Models for replication-coupled nucleosome assembly (RCNA) highlighting old histone H3–H4 recycling.
(**A**) In *Drosophila* male GSCs, old $(H3–H4)_2$ are preferentially recycled to the leading strand with yet-to-be identified mechanisms [12]. By default, newly synthesized $(H3–H4)_2$ are incorporated by the lagging strand, thereby generating a biased histone incorporation in a strand biased manner. (**B**) In mESCs, the role of Mcm2 is shown to recycle old $(H3–H4)_2$ towards the lagging strand [66]. (**C**) In yeast, old histones recycle towards leading strand via interactions with Dpb3/Dpb4-Polε [68], while the MCM2-ctf4-Polα axis is shown to recycle old $(H3–H4)_2$ towards the lagging strand [67].

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(Proliferating Cell Nuclear Antigen), a processivity factor for DNA polymerases [70], coordinates histone deposition at the replication fork [71,72]. In addition, CTF4, Pol α and PCNA were found enriched at the lagging strand [73], leading to the hypothesis that this axis could serve to deposit old histones onto the lagging strand (Figure 1C) [72,74]. Recently, the Mcm2–Ctf4–Pol α axis has been found to facilitate the re-distribution of parental H3–H4 tetramers to lagging-strand DNA at replication forks (Figure 1C) [67]. Consistent with the results in mESCs, mutating the histone-binding domain of the Mcm2 in yeast leads to an enrichment of parental H3–H4 on the leading strand. Similar effects were also observed with the Ctf4 and Pol α primase mutants (Figure 1D) [67].

Regarding the dynamics of nucleosomes during the RCNA process, it has been shown that most of the old $(H3–H4)_2$ tetramers remain intact during DNA replication. This suggests that nucleosomes contain either old tetramers or new $(H3–H4)_2$ tetramers, rather than a tetramer containing both [75,76]. In contrast, most of the old $(H2A–H2B)_2$ are split as dimers, suggesting that the newly formed nucleosomes should contain a mixture of old and new H2A–H2B, rather than solely one or the other. Therefore, the old intact $(H3–H4)_2$ tetramer can only be inherited by one strand (either the leading or lagging strand), leading to more asymmetric inheritance patterns at the replication fork. On the other hand, the presence of two split old H2A–H2B dimers gives an equal probability of being incorporated by either the leading or lagging strand, thus resulting in a tendency of more symmetric inheritance pattern. In addition to the splitting mode differences, the $(H3–H4)_2$ tetramers are relatively more stable in the nucleosome, while the (H2A–H2B) dimers display a more rapid exchange during the post-replication chromatin maturation process [77–79]. This could also explain the differential distribution patterns seen on replicated sister chromatids that enter mitosis.

Additionally, an imaging-based technique named Super-Resolution of Chromatin Fiber (SRCF) has been developed to directly visualize distribution of old *versus* new histones at the replication regions by combining high spatial resolution imaging and chromatin fiber techniques [12,80]. Using early germline-derived chromatin fibers that express old and new H3 or H4 labeled with two distinct fluorescent tags, the recycling pattern of the old and new H3 or H4 at the replication regions can be resolved. SRCF revealed that the old and new histones H3 and H4 are recycled with a leading strand bias, whereas new H3 and H4 are incorporated towards the lagging strand (Figure 1A). In contrast, both old and new histones H2A showed a largely symmetric incorporation pattern at the replication fork [12]. Such differential histone incorporation patterns indicate the presence of molecular specificities during RCNA [11,12,81–83] Furthermore, DNA fibers that are sequentially labeled by the thymidine analog EdU and BrdU facilitate the investigation of directionality in replication fork movement. Using symmetrically dividing somatic cells as a control, a significantly higher incidence of unidirectional fork movement was detected in early germ cells. The strand biased old *versus* new histone incorporation, coupled with predominantly unidirectional fork movement, could act together to establish an asymmetric histone incorporation pattern in GSCs. While the SRCF is a powerful technique that is compatible with a few cells such as adult stem cells, improvement on cell type and cell stage specificities is needed to label or isolate fibers precisely from stem cells. Future studies will help further our understanding of the molecular mechanisms underlying such a biased incorporation pattern in the *Drosophil*a male GSC system. Consistent with the asymmetric old and new H3 and H4 incorporation during DNA replication, such asymmetry is reflected by a global difference between sister chromatids during and after *Drosophila* male GSC asymmetric divisions (Figure 2A). Conversely, old and new H2A that show symmetric incorporation at replication regions display largely symmetric inheritance patterns during and after ACD of male GSCs. In summary, these results demonstrate that the distinct histone incorporation patterns at the replication fork underlie their different inheritance modes, as observed in asymmetrically dividing stem cells and post-mitotic daughter cells. Therefore, DNA replication establishes the asymmetry of the epigenome. In contrast, such an asymmetric histone inheritance pattern is not detected in the symmetrically dividing progenitor germ cells, indicating that the processes of RCNA are likely subject to cell type or stage-specific regulation during tissue homeostasis.

Distinguishment of asymmetric epigenome

Centromere function: from genome segregation to cell fate determination

The centromere is a chromosomal region where the kinetochore protein complexes assemble during M phase [84,85]. Spindles attach to the kinetochore and segregate sister chromatids into each of the daughter cells [86]. Centromeres are epigenetically defined by CENP-A in mammals [15–19] (called Centromere Identifier (CID) in *Drosophila* [87]), which is necessary and sufficient for centromere identity and function. Centromeres have

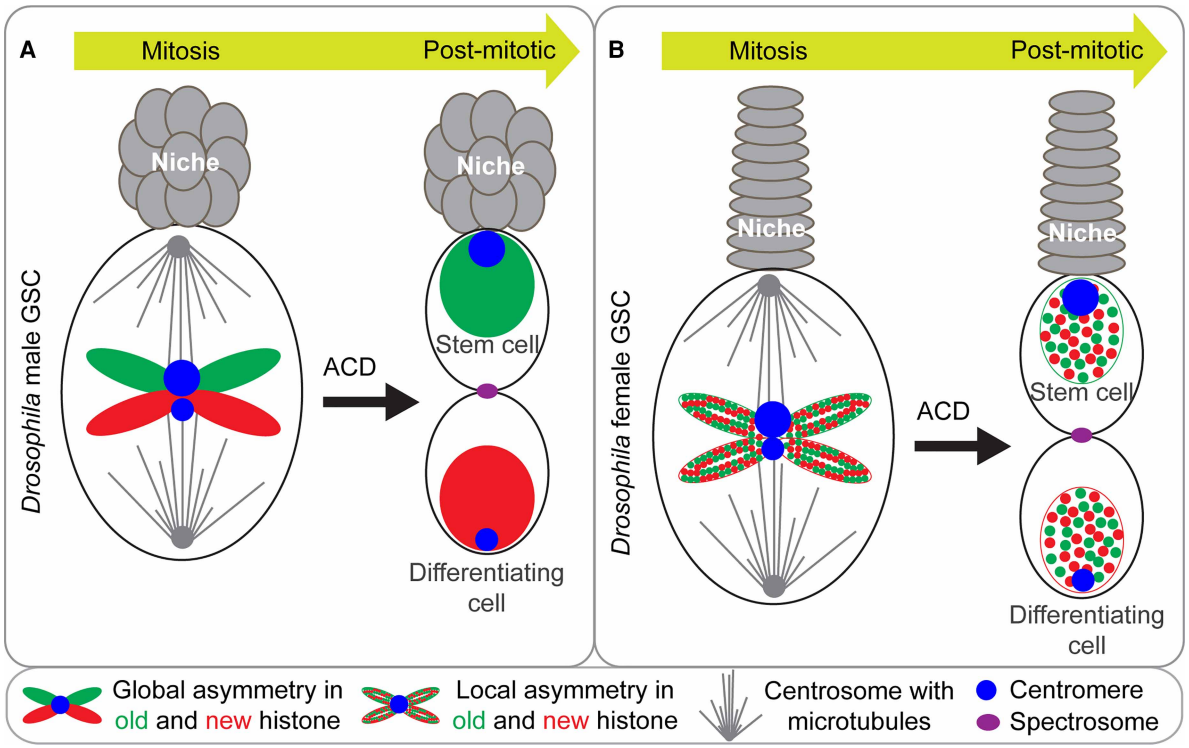

Figure 2. Schematic illustration of the different histone inheritance pattern in different stem cell system.

(**A**) *Drosophila* male GSC: sister chromatids are either enriched with old histone (green) or new histone (red) H3 and H4. Histone segregates asymmetrically into the daughter cells; a representative example of global histone asymmetry. Also, CENP-A segregates asymmetrically into daughter cell, represent centromere epigenetic asymmetry and non-random sister chromatids segregation. (**B**) *Drosophila* female GSC: sister chromatids are overall carrying a symmetric level of old and new histone. However, a local asymmetry between old and new histone can be seen at stem cell maintenance genes and differentiation gene loci; a representative example of gene-specific histone asymmetry (also found in mouse embryonic stem cells that are induced to undergo ACD). Interestingly, despite of the overall symmetric histone segregation, CENP-A segregates asymmetrically into daughter cell, suggesting sister chromatids still carry epigenetic information differentially and segregate non-randomly.

been discovered for over a century as a specific chromosomal region that regulates the partition of sister chromatids [88], but their molecular identity has only recently been documented [15,87,89]. For example, a recent study in *Drosophila* combines long-read sequencing, chromatin immunoprecipitation of CID/CENP-A protein, and chromatin fiber imaging, revealing that the centromeres form on islands of complex DNA sequences enriched in retroelements flanked by large arrays of satellite repeats [89]. More recently, the human centromere sequence has been investigated using oligo-FISH, CRISPR-based experiments, and the newly developed NTRprism, a versatile algorithm for discovering and visualizing satellite repeat periodicity. This study has revealed novel patterns of centromeric repeat organization, variation, and evolution at both large and small length scales [90]. These recent discoveries provide an unprecedented atlas of centromeres to guide future studies regarding their biological functions and evolutionary dynamics. Centromeres are well known for their critical roles in the faithful segregation of the duplicated genome equally into the two daughter cells during conventional symmetric cell divisions [91–96]. However, its roles during ACD in segregating non-genetic components and cell fate determination is an emerging area yet to be explored. Here, we discuss recent discoveries on the functions of centromeres in epigenetic inheritance and cell fate determination during ACD in different systems.

Asymmetric sister centromeres: the key to the ‘mitotic drive’ phenomenon

Many reports have shown that sister chromatids segregate non-randomly during ACD, suggesting its relationship with distinct cell fate determination [27,97–101]. However, the underlying mechanisms of how mitotic

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https://doi.org/10.1042/BST20200267

machinery could distinguish the potential epigenetic differences between sister chromatids to ensure non-random segregation remained poorly understood. Sister centromeres have been proposed to differ epigenetically [102], which would contribute to the asymmetric inheritance of other epigenetic information carried by sister chromatids. However, this hypothesis has been largely unexplored due to the lack of a paradigmatic system for a clear readout between sister centromeres with a distinct inheritance pattern.

It has been debated whether the levels of CENP-A could contribute to cell fates. Human pluripotent stem cells (hPSCs) have been shown to have a unique reserve of CENP-A mRNA which is not found in differentiated fibroblasts [103]. However, despite having a higher level of mRNA in hPSCs, the amount of CENP-A protein is similar in hPSC as compared with fibroblasts. Surprisingly, no significant phenotypes were observed upon depletion of *CENP-A* mRNA or CENP-A protein in hPSCs when cultured with self-renewal-promoting conditions. In contrast, a significant increase in apoptosis was observed by inducing differentiation of the CENP-A-depleted hPSCs [103]. These results suggest that the CENP-A levels play an important role in the process of cellular differentiation. A study on the midgut epithelium of *Drosophila*, where ISCs reside, revealed that *CENP-A* mRNA levels almost doubled in ISCs as compared with differentiated enterocytes (ECs); however, it is significantly increased in terminally differentiated enteroendocrine (EEs) compared with ISCs [21]. Taken together, these results suggest that the amount of CENP-A protein is cell type- and developmental context-specific. Additionally, when the CENP-A protein was analyzed, the pre-existing old CENP-A and the newly synthesized CENP-A were shown to segregate asymmetrically. The old CENP-A was preferentially inherited by the self-renewed ISCs and was retained in ISCs for many cell cycles. In contrast, new CENP-A is predominantly inherited by the enteroblasts (EBs), the differentiation daughter cells derived from ACD of ISCs [21]. Furthermore, in male *Drosophila* GSCs, old CENP-A segregates asymmetrically, leading to old CENP-A enrichment in the self-renewed stem cell [20]. While the old CENP-A recycles in a DNA replication-dependent manner, the new CENP-A incorporation is independent from replication and has been shown to be incorporated between mid G2 to early prophase. Unlike new CENP-A incorporation during G1 phase in human HeLa cells [104] but similar to new CID incorporation in *Drosophila* female GSCs [21]. Notably, it has been shown that new CENP-A incorporation is dispensable for mitotic centromere function after kinetochore assembly [105]. Therefore, incorporating new CENP-A prior to spindle attachment may indicate its role during ACD. Together, these results suggest that the nature of the epigenetic difference between sister centromeres, for example, old *versus* new CENP-A and their potentially different PTMs, may contribute to cell fate determination during and after ACD.

While sister centromeres are supposed to have identical DNA sequences, it has been found that sister centromeres differ in their CENP-A protein levels in *Drosophila* male GSCs. One sister centromere has more CENP-A protein than the other sister centromere, creating a quantitative difference between the pair of sister centromeres [11,20]. Moreover, the sister centromere with more CENP-A protein is inherited by the self-renewed stem cells, whereas the sister centromere with less CENP-A protein is inherited by the differentiating daughter cells. These results suggest that sister centromeres segregate non-randomly during ACD of GSCs. Disruption of sister centromere asymmetry by compromising CAL1, the chaperone that assists incorporation of new CENP-A, results in the loss of sister centromere asymmetries and subsequently symmetric CENP-A segregation. These results suggest that CAL1 is required for the asymmetric sister centromeres detected in the mitotic GSCs. Crucially, knocking down CAL1 also results in stem cell loss phenotypes in *Drosophila* testes [11,20]. Together these results indicate two key points: (1) sister centromeres have different CENP-A protein levels in asymmetrically dividing stem cells, and (2) epigenetically distinct sister centromeres are segregated non-randomly during ACD of stem cells. Together, these findings provide the first direct evidence that sister centromeres carrying distinct epigenetic features can be recognized and inherited differentially in asymmetrically dividing stem cells, which are likely responsible for the distinct fates of the resulting daughter cells.

Intriguingly, Datolli et al. identified similar asymmetric sister centromeres in *Drosophila* female GSCs. Disruption of asymmetric sister centromeres could be achieved by inactivating CAL1 or compromising CENP-A, which results in female GSC maintenance defects [106]. In addition, both the inner kinetochore protein CENP-C in female GSCs [106] and the outer kinetochore protein NDC80 in male GSCs [20] assemble asymmetrically, with an even higher degree of asymmetry than in CENP-A between sister centromeres in female and male GSCs, respectively. The commonalities between male and female GSC systems in *Drosophila* suggest the existence of a relay mechanism from asymmetric sister centromeres to sister kinetochores. Furthermore, depletion of CENP-C disrupts the incorporation of the new CENP-A in *Drosophila* female GSCs, suggesting that CENP-C helps assemble asymmetric sister centromeres [107]. Additionally, the knockdown of either CENP-A or CENP-C in the *Drosophila* midgut results in decreased proliferative ISCs [21]. Taken together, these results suggest that the asymmetric epigenetic features between sister centromeres could be a common phenomenon in asymmetrically dividing cells.

In summary, recent studies have shown that sister centromeres have epigenetic differences, as hypothesized previously [102], raising the question of whether these differences could drive non-random sister chromatid segregation. Garcia et al. showed that in *Drosophila* ISCs, compromising CENP-A, CAL1, or CENP-C results in decreased ISCs proliferation, while CAL1 depletion results in ISC loss. Furthermore, Ranjan et al. showed that in *Drosophila* male GSCs, inactivation of CAL1 results in the symmetric sister centromeres with comparable CENP-A levels, leading to GSC loss. Consistently, in *Drosophila* female GSCs, Datolli et al. demonstrated that the overexpression of both CENP-A and CAL1 may lead to a symmetric distribution of CENP-A between sister chromatids in early germ cells, including GSCs and CBs, which results in a shift towards stem cell self-renewal and the accumulation of GSC-like cells. Together, these results reveal that the manipulation of sister-centromere asymmetry leads to defects in stem cell maintenance and proper differentiation of the differentiating daughter cells. Moreover, these studies demonstrate that stem cells require asymmetric sister centromeres to direct non-random segregation of sister chromatids.

Mitotic machinery distinguishes epigenetic differences between sister centromeres

Centrosomes are the microtubules organization center (MTOC) in a cell. It is interesting to note that asymmetric inheritance of centrosomes has been reported during ACD of different stem cell types. For example, *Drosophila* male GSCs and mouse neural glial progenitor cells inherit the mother centrosome, while the differentiating daughter cell inherits the daughter centrosome [22,25]. On the other hand, *Drosophila* female GSCs and neuroblasts inherit the daughter centrosome, and the differentiating daughter cells inherit the mother centrosome [23,24]. Such a biased inheritance of age-different centrosomes provokes the speculation that non-random sister chromatids segregation is tightly coordinated with differential centrosome inheritance. Questions remained, for example, is there a differential crosstalk between the microtubule nucleation from mother *versus* daughter centrosomes and epigenetically distinct sister centromeres? Because microtubule-centromere interactions are highly dynamic, a high spatial and temporal resolution live cell imaging approach is necessary to study their communications. However, live cell imaging of the mitotic machinery components in cells from intact tissue with high spatiotemporal resolution has been technically challenging [108]. Recently, a Super-Resolution Live Snapshots (SRLS) method has been developed to visualize mitotic spindle and sister centromere dynamics at a high spatial resolution in intact tissues [109]. High spatial resolution snapshots of living cells were collected and arranged according to their sequential orders during cell cycles to investigate highly dynamic cellular events in detail. Using SRLS, it has been shown that the MTOC activity between mother and daughter centrosomes is temporally asymmetric in *Drosophila* male GSCs. The mother centrosome is highly active during the late G2 phase, prior to the daughter centrosome, which only becomes active during the G2-to-M phase transition (Figure 3A). These temporally asymmetric microtubules lead to a polarized nuclear membrane breakdown (Figure 3B), which happens first at the stem cell side during the G2–M transition and later at the differentiating daughter side in prometaphase GSCs (Figure 3C). These data suggest that asymmetric microtubule activity and polarized nuclear membrane breakdown work in tandem to bias microtubule-kinetochore attachment. First, microtubules emanated from the mother centrosome on the stem cell side preferentially attach to the stronger sister centromere during prophase (Figure 3B). Later, microtubules emanated from the daughter centrosome on the differentiating daughter side subsequently attach to the weaker sister centromere during prometaphase (Figure 3C) [20]. It is worth noting that the stronger sister centromere, together with the stronger sister kinetochore, show higher affinities with microtubules, leading to more microtubule attachment (Figure 3B–D). This differential attachment is likely stabilized, resulting in non-random sister chromatid segregation as observed in both *Drosophila* male and female GSCs (Figure 2A,B) [20,106]. Therefore, the mitotic machinery forms an asymmetric axis in a spatiotemporally regulated manner during ACD of GSCs as follows: centrosome > microtubules > nuclear membrane > kinetochore > centromere. This ensures that the sister chromatid inheritance will be nonrandom. On the other hand, disruption of the temporally asymmetric microtubules by nocodazole, a microtubule depolymerizing drug, results in the loss of preferential microtubules and sister centromere attachment, and subsequent randomized sister chromatid segregation [20]. These discoveries demonstrate that a cascade of tightly coordinated spatially and temporally asymmetric *cis* factors (e.g. sister centromeres) and *trans* events (e.g. microtubules) are essential for asymmetric epigenetic inheritance and cell fate differences. These highly coordinated events in asymmetrically dividing cells have been termed as 'mitotic drive' [11,20] (Figure 3 and Supplementary Movie 1).

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https://doi.org/10.1042/BST20200267

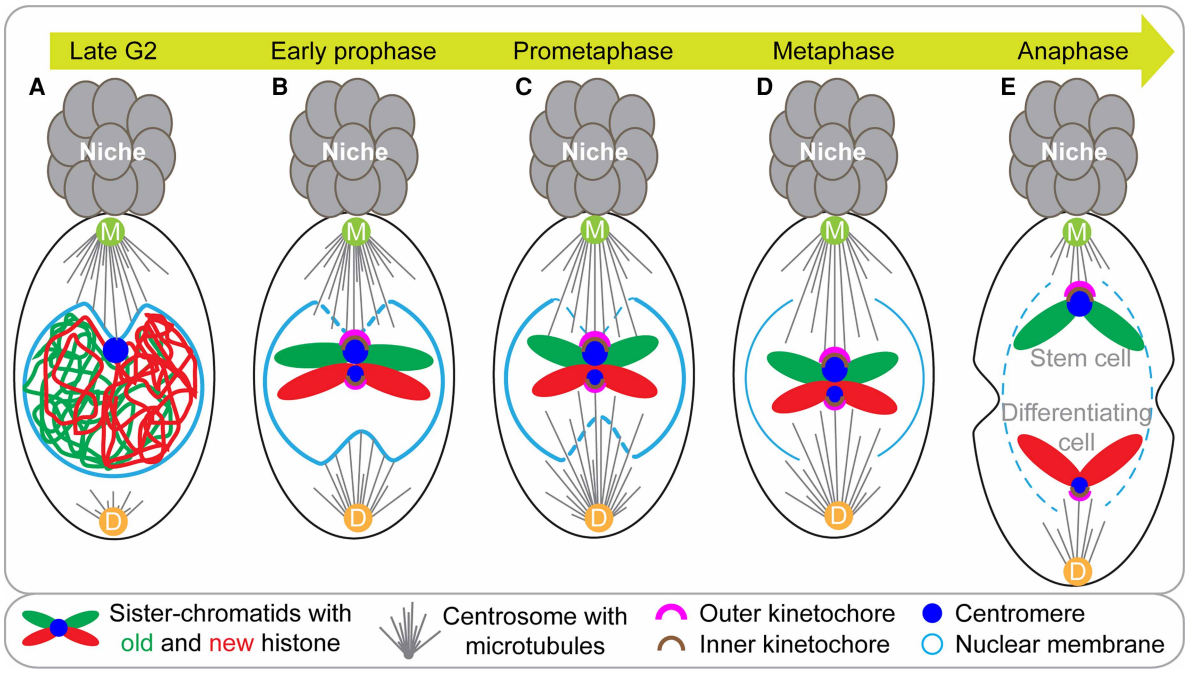

Figure 3. Mitotic drive phenomenon in *Drosophila* male GSCs.

Schematic diagram showing one pair of sister chromatids. (**A**) Late G2: active microtubules from the mother centrosome start poking nuclear membrane toward the stem cell side. At the same time, centromeres are clustered at the stem cell side where microtubules poke in. However, sister centromeres are not resolved at this time. (**B**) Early prophase: highly active microtubules from the mother centrosome locally break the nuclear membrane toward the stem cell side, allowing microtubules to enter into the nucleus. The stronger sister centromere is stably attached with more microtubules. At the same time, daughter centrosome nucleates active microtubule that start poking nuclear membrane toward the differentiating daughter cell side. (**C**) Prometaphase: active microtubules emanated from the daughter centrosome locally break the nuclear membrane toward the differentiating daughter cell side. The weaker sister centromere is attached by daughter centrosome-emanating microtubules. (**D**) Metaphase: a biased bi-oriented spindle with the stronger centromere attached by the mother centrosome-emanating microtubules, whereas the weaker centromere attached by the daughter centrosome-emanating microtubules. (**E**) Anaphase: the biased microtubule-kinetochore attachment leads to non-random sister chromatid segregation, the stronger centromere at the old histone enriched-chromatid segregates to the self-renewing stem cell, whereas the weaker centromere at the new histone-enriched chromatid is segregated into the differentiating daughter cell side.

A 'meiotic drive' phenomenon has been reported in meiosis I during the development of a mouse egg [110,111]. This observation is called 'centromere drive' when centromeres serve as a 'selfish' element. When mice strains with different kinetochore strengths breed, the oocyte inherits the stronger kinetochore and centromere while the weaker kinetochores and centromeres are inherited by the polar bodies, which subsequently degenerate [112–114] Here, the stronger centromeres have 6 to10 fold more minor satellite repeats, allowing them to recruit more kinetochore proteins. In addition, the meiotic spindle shows asymmetric PTMs, such as high tyrosinated microtubules towards the polar body side compared with the egg side due to the polarized CDC42 signaling [115]. This spindle asymmetry and polarized cortical CDC42 signal were found to be essential to flip and re-orient the stronger centromere towards the egg side. These results suggest that the polarized cortical CDC42 signals serve as a spatial cue for selfish centromeres to distinguish between egg and polar bodies to ensure their preferential retention during meiosis I. Furthermore, these studies show that the flipping and reorientation events of centromeres on homologous chromosomes depends on the amount of destabilizers, such as chromosome passenger complex (CPC) and mitotic centromere-associated kinesin (MCAK) [116]. Stronger centromeres recruit more destabilizing factors (MCAK and CPC), increasing the opportunity of the stronger centromeres to flip, especially when they are attached with tyrosinated microtubules. In addition, 'meiotic drive' might involve non-centromeric loci, such as the knob domain in Maize, which acts as a neocentromere by attaching to the meiotic spindle without a kinetochore, leading to the

Table 1 Comparison of meiotic drive (centromere drive) and mitotic drive

Factors involve	Centromere drive (meiotic drive)	Mitotic drive
Chromosomal feature	Between homologous chromosomes	Between sister chromatids
Centromere feature	Inherent asymmetry in centromere length	Theoretically symmetric centromere length
Reason for centromere asymmetry	Genetic: due to greater number of minor satellite repeats	Epigenetic: due to quantitative asymmetry in CENP-A
Frequency of occurrence	Occasionally: when mouse lines with distinct centromere size breed	Almost always: when stem cell undergoes asymmetric division
Biological significance	Karyotype evolution	Tissue homeostasis
Structural asymmetries involved	Post-translational modification asymmetry in microtubules	Temporal and quantitative asymmetry in microtubules
Centromere orientation mechanisms	Uses destabilizers to flip and reorient centromeres, e.g. MCAK and CPC	Remain elusive
Involvement of non-centromeric regions	Heterochromatin containing repeats act as a neocentromere, e.g. knob domain in Maize	Remain elusive

preferential retention of knob domain chromosomes in progenies [110,117,118]. 'Meiotic drive' and 'mitotic drive' each have distinct features, which are summarized in Table 1 and in recent reviews [11,119].

Histone inheritance modes in other systems

After the initial discovery in the *Drosophila* male GSC system, it became of great interest to examine the generality of this phenomenon and how it may regulate cell fate decisions in different systems and developmental contexts. Recently, it has been reported that global asymmetric histone inheritance also occurs in asymmetrically dividing *Drosophila* ISCs [120]. Similar to the male GSCs, old H3 and H4 are predominantly inherited by the self-renewed ISCs, while new histones are inherited by the differentiating daughter cells. In contrast, such asymmetric inheritance of old and new H3 and H4 is not observed when ISCs undergo symmetric division, suggesting that asymmetric inheritance is contingent with distinct daughter cell fates. However, global asymmetric histone inheritance was not observed in *Drosophila* female GSCs. Instead, a local asymmetry between old and new H3 and H4 was detected. Using a novel approach combining Oligopaint DNA FISH with dual-color histone labeling to study gene-specific histone inheritance patterns, a differential distribution of old *versus* new H3 at the key genes for either maintaining the stem cell state or for promoting differentiation was revealed. This differential distribution has both cellular specificity for early-stage GSCs or cystoblasts but not for late-stage cystocytes and molecular specificity for H3 but not H2A [121]. This suggests that a gene-specific or domain-specific regulation exists in this system (Figure 2B). Such local asymmetry in histone inheritance was also observed in mouse embryonic stem cells (mESCs) when ACD was induced with Wnt3a-coated beads [122]. It is plausible that the asymmetry between global versus local scale relies on the number of differentially expressed genes that are turned on or switched off during stem cell differentiation. For example, in the case of *Drosophila* male GSCs, a large number of genes needs to be turned on or off to regulate spermatogenesis, which could be the cause of global histone asymmetry [123]. Whereas in the *Drosophila* female GSCs, a smaller subset of genes is required to turn on or off for oogenesis. Such differences in the gene expression landscape could be a contributing factor in global *versus* local histone asymmetry patterns. A key area of future research is to better understand the factors involved in regulating global *versus* local asymmetric histone inheritance observed in different stem cell systems as well as their potentially distinct biological consequences.

Perspectives

- Epigenetic memories play a crucial role in defining cellular identities during development and to maintain tissue homeostasis, therefore, mis-regulation of epigenetic inheritance leads to cell fate determination defects, resulting in diseases such as cancer, tissue dystrophy, infertility, as well as ageing.

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https://doi.org/10.1042/BST20200267

- Studies show that DNA replication components have histone chaperone activities to regulate the distribution of old *versus* new between sister chromatids [66–68,124]. Sister centromeres have epigenetic differences to ensure asymmetric segregation of sister chromatids, which could lead to distinct daughter cell fates during asymmetric stem cell division in multiple systems [11,20,106].

- It is important to understand how and when asymmetric centromeres are established and the generality of these phenomena for future research.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contributions

R.R. conceptualized and wrote the manuscript and X.C. edited the manuscript.

Acknowledgements

We apologize to those whose related publication could not be cited due to space limitations. We are grateful to all members of Chen's Lab for insightful comments and suggestions. Supported by NIGMS/NIH R35GM127075, NICHD/NIH R01HD102474, the Howard Hughes Medical Institute Faculty Scholar and Investigator programs (X.C.), the David and Lucile Packard Foundation, and Johns Hopkins University startup funds (X.C.).

Abbreviations

ACD, asymmetric cell division; CENP-A, CENtromere Protein A; CID, Centromere Identifier; CPC, chromosome passenger complex; GSCs, germline stem cells; ISCs, intestinal stem cells; MCAK, mitotic centromere-associated kinesin; MTOC, microtubules organization center; PCNA, Proliferating Cell Nuclear Antigen; PTMs, post-translational modifications; RCNA, replication-coupled nucleosome assembly; SRCF, super-resolution of chromatin fiber; SRLS, super-resolution live snapshots.

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