Symmetry from Asymmetry or Asymmetry from Symmetry?

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The processes of DNA replication and mitosis allow the genetic information of a cell to be copied and transferred reliably to its daughter cells. However, if DNA replication and cell division were always performed in a symmetric manner, the result would be a cluster of tumor cells instead of a multicellular organism. Therefore, gaining a complete understanding of any complex living organism depends on learning how cells become different while faithfully maintaining the same genetic material. It is well recognized that the distinct epigenetic information contained in each cell type defines its unique gene expression program. Nevertheless, how epigenetic information contained in the parental cell is either maintained or changed in the daughter cells remains largely unknown. During the asymmetric cell division (ACD) of *Drosophila* male germline stem cells, our previous work revealed that preexisting histones are selectively retained in the renewed stem cell daughter, whereas newly synthesized histones are enriched in the differentiating daughter cell. We also found that randomized inheritance of preexisting histones versus newly synthesized histones results in both stem cell loss and progenitor germ cell tumor phenotypes, suggesting that programmed histone inheritance is a key epigenetic player for cells to either remember or reset cell fates. Here, we will discuss these findings in the context of current knowledge on DNA replication, polarized mitotic machinery, and ACD for both animal development and tissue homeostasis. We will also speculate on some potential mechanisms underlying asymmetric histone inheritance, which may be used in other biological events to achieve the asymmetric cell fates.

Asymmetric inheritance of cell fate determinants in developing organisms is known to play a major role in cellular differentiation, and it is a fundamental process in generating cellular diversity. Our current understandings of the mechanisms that orchestrate asymmetric cell division (ACD) have been gathered from a wide variety of developmental model organisms, including yeast, flies, worms, and mice, among others. As early as 1905, cell lineage analysis of the ascidian *Styela partita* identified cytoplasmic determinants derived from the egg that segregate to distinct cell lineages responsible for generating five specialized tissue types (Conklin 1905). Despite examples of intrinsic segregation of cell fate determinants, it was not until 1994 that the first determinant, Numb, was molecularly characterized (Rhyu et al. 1994). To date, key determinants of cell fate found to be distributed unequally in ACDs include cell surface receptors, transcription factors, mRNA, DNA, histones, and organelles such as endosomes, centrosomes, and mitochondria (Carmena 2008; Knoblich 2008; Tran et al. 2013; Katajisto et al. 2015). During development, this asymmetry is critical for generating divergent cell fates and progenitor cell self-renewal. Failure of these mechanisms can lead to severe defects in cell proliferation, which manifest as tissue degeneration or tumorigenesis.

The asymmetric inheritance of DNA molecules as a cell fate determinant during ACD has been considered previously. In 1975, John Cairns proposed the “immortal strand” hypothesis, suggesting that the stem cell continu-

ally inherits the old DNA strands to minimize accumulation of random DNA replication errors that could change cell fate (Cairns 1975). However, the immortal strand hypothesis has not been widely accepted because of the lack of solid supporting *in vivo* evidence. Two similar (and more accepted) models, named the “strand-specific imprinting and selective chromatid segregation” (Klar 1994, 2007) and “silent sister chromatid” (Lansdorp 2007) hypotheses suggest epigenetic differences between sister chromatids are required to direct the asymmetric outcomes during ACD.

In this review, we will discuss how the processes of DNA replication, chromosomal segregation, and cell division lead to asymmetric outcomes and how organisms are able to develop, maintain homeostasis, and adapt to a changing environment through these asymmetric processes. We argue that the symmetric outcome of making exact copies of DNA and daughter cells is necessary but not sufficient for the propagation and diversification of life. We then hypothesize that the development and homeostasis of multicellular organisms depend on modified molecular and cellular processes to generate asymmetry from the mechanisms that control the otherwise equal distribution of cellular components into the two daughter cells. We will discuss studies that have reported on asymmetric inheritance of cell fate determinants in diverse organisms with a focus on epigenetic differences between sister chromatids, and we will give examples of nonrandom segregation of sister chromatids.

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DNA REPLICATION IS AN ASYMMETRIC PROCESS THAT CAN BE BIASED

The asymmetric outcomes of DNA replication and cell division rely heavily on modifications that lead to heritable changes in gene expression and, hence, cell fate. Such modifications occur without altering the primary sequence of the DNA and are collectively referred to as epigenetics (Jacobs and van Lohuizen 2002; Turner 2002; Ringrose and Paro 2004; Probst et al. 2009). It is possible that DNA replication has a heretofore underappreciated role in establishing distinct epigenomes between sister chromatids that will be inherited by each daughter cell upon cell division. DNA consists of two antiparallel strands containing a deoxyribose sugar–phosphate backbone that supports varying sequences of four bases that pair in a complementary way. Through elegant studies, we know that DNA is synthesized in a semiconservative manner, meaning that each daughter DNA will inherit one template strand and one newly synthesized strand as double-stranded DNA (dsDNA) (Meselson and Stahl 1958).

The components of DNA replication machinery bind to DNA in pairs and initiate DNA replication in a bidirectional manner. Because DNA can only be synthesized in the 5'→3' direction, the DNA polymerase responsible for creating the new strand is required to read the single-stranded (ss) template in the 3'→5' direction, beginning from an existing 3'-OH overhang. Interestingly, this creates an inherent asymmetry as to how the new strands are synthesized. One strand, the leading strand, begins with a single RNA primer and can be synthesized continuously as the advancing replication fork exposes more ss template and the template is read in the 3'→5' direction (Bessman et al. 1956, 1958; Lehman et al. 1958; Meselson and Stahl 1958; Kornberg et al. 1989). However, the other template strand, termed the lagging strand, runs antiparallel to the leading strand and cannot be read by the polymerase in the same direction as the advancing replication fork. Thus, the lagging strand is synthesized in short segments, called Okazaki fragments, in the direction opposite to the advancing replication fork. Each Okazaki fragment begins with an RNA primer and is, in fact, synthesized by DNA polymerases different from those of the leading strand (Sakabe and Okazaki 1966; Okazaki et al. 1968; Balakrishnan and Bambara 2013). Furthermore, the lagging strand must undergo additional processing to remove ss “flaps” left behind. To explain, DNA polymerase δ displaces nucleotides from the previously synthesized DNA polymerase α fragment, and nicks left between fragments must be sealed by DNA ligase (Table 1, Fig. 1; Bambara et al. 1997; Rossi et al. 2008; Cerritelli and Crouch 2009).

Although much is known about replication fork licensing and elongation, it is interesting to consider that the origins of replication have yet to be well defined within most eukaryotes, and that transcription can have a direct effect on the localization of prereplication complexes (Vashee et al. 2003; Cayrou et al. 2011). For each cell type, the transcriptional machinery may affect the density and location of replication origins and the length of the replicons in between them and may bias the replication

Table 1. Leading- versus lagging-strand enriched molecules with their function in brief

Replication component	Strand enrichment	Function
DNA polymerase ϵ	Leading	Synthesizes the leading strand
MCM2–7 helicase	Leading	Unwinds DNA for replication
Cdc45	Leading	Interacts with Mcm proteins; converts the prereplicative complex to the initiation complex
GIN5	Leading	Essential for the interaction of Mcm proteins and Cdc45 during initiation and elongation
MCM10	Leading	Activates the Cdc45–MCM–GIN5 helicase at DNA replication origins
DNA polymerase α	Lagging	Begins replication by synthesizing an RNA primer and adding approximately 20 DNA nucleotides
DNA polymerase δ	Lagging	Synthesizes the lagging strand
PCNA	Lagging	Ring-shaped clamp that stabilizes DNA polymerases onto DNA
RFC	Lagging	Loads PCNA onto the DNA
RPA	Lagging	Binds ssDNA to prevent secondary structure formation
RNase H	Lagging	Removes any remaining RNA nucleotides
DNA2 and FEN1	Lagging	Remove “flaps” of DNA created by DNA Pol δ advancing into and lifting the previous Okazaki fragment
Ligase	Lagging	Seals nicks in the DNA backbone between segments of newly synthesized DNA

Data from Langston et al. 2014; Yu et al. 2014.

of certain genes to either the leading or the lagging strand. Known fork-blocking proteins could also serve to bias the length and direction of replication forks. Paradoxically, although transcription may affect DNA replication, DNA replication is also likely to affect transcription. That is, transcription machinery is displaced as DNA is unwound and must rebind following fork passage. Now, however, it can only bind one of the two copies of DNA present after replication. Studies have shown that rebinding of the transcription machinery can be biased to either the leading or the lagging strand, depending on the rate of fork progression and the inherent maturation of the two strands after fork passage (Alabert and Groth 2012; Vasseur et al. 2016). It has also been shown that this biased rebinding event can lead to heritable changes in gene expression where one daughter cell “remembers” its transcriptional state and the other daughter cell lags behind, with the need to reestablish its transcriptional state (Ferraro et al. 2016).

HISTONE RECYCLING AFTER DNA REPLICATION COULD BIAS CELL FATE

In addition to the inherent asymmetries between the leading and lagging strands of DNA replication, one

Figure 1. A schematic cartoon of DNA replication fork. DNA replication, although intended to create two equal copies of a double-stranded DNA template, does possess inherent asymmetries relative to strand synthesis. Indeed, several experiments have found functional outcomes that arise from asymmetric leading- versus lagging-strand synthesis. For example, the discontinuous synthesis of the lagging strand has been postulated as a more error-prone process. This could potentially lead to an increased mutation rate that may be evolutionarily beneficial without compromising the genomic stability of the continuously synthesized leading strand (Furusawa 2014). Additionally, it has been shown that molecular lesions created during lagging-strand synthesis contribute to mating type switching in both the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* (Hanson and Wolfe 2017).

must also consider asymmetries in the epigenetic modification of the DNA itself, as well as the nucleosome, the basic packaging unit of DNA. Methylation of DNA has been well studied and is generally associated with transcriptional repression. Just as with the transcriptional machinery, recovery of DNA methylation appears more slowly on the lagging strand than it does on the leading strand, perhaps allowing time for the two sisters to be differentially recognized or for the methylome on the lagging strand to be rewritten (Stancheva et al. 1999; Tajbakhsh and Gonzalez 2009).

Another major epigenetic information carrier for cell fate is the nucleosome structure, which is comprised of eight histone proteins (two H2A–H2B dimers and one H3–H4 tetramer). Posttranslational modifications of histone proteins have profound effects on cell fate and transcriptional activity (Peterson and Laniel 2004). Of note, nucleosomes must be disassembled ahead of the replication fork and reassembled onto one of the two new dsDNA templates that now exist in the wake of the fork (McKnight and Miller 1977; Sogo et al. 1986). Although the process of new histone deposition onto the DNA has been well studied, how preexisting histones are recycled during DNA replication is less clear (Burgess and Zhang 2013). Elucidating this mechanism is essential to understanding how DNA replication may impact epigenetic information partitioning.

To date, three possible models of histone recycling after fork progression have been proposed. First, the semiconservative model suggests that the H3–H4 tetramer is split into two dimers such that the four dimers of the nucleosome (two H2A–H2B and two H3–H4) are evenly distributed between the two new dsDNA strands. This mechanism was thought to be an elegant solution to evenly distributing epigenetic information such that both daughter strands would inherit equal posttranslational histone modifications, predominantly carried by the H3 and H4 tails (Zhu and Reinberg 2011). However, several lines of evidence have surfaced against the semiconservative

model of histone recycling. For example, it has been found that the H3–H4 tetramer rarely, if ever, splits into two dimers once the tetramer has been assembled (Xu et al. 2010). Furthermore, the tails of H3 and H4 within each tetramer are not symmetrically modified (Chen et al. 2011; van Rossum et al. 2012; Voigt et al. 2012). Thus, even if the tetramer does split, with each new dsDNA inheriting one H3–H4 dimer, then the epigenetic information of the previously unreplicated region would not be preserved equally between the two daughter strands (Fig. 2A). Second, the dispersive model of histone recycling proposes that the H3–H4 tetramer remains intact, but the tetramers and dimers disassembled ahead of the fork are still randomly distributed between the leading and lagging strands behind the fork (Jackson and Chalkley 1981, 1985; Alabert and Groth 2012; Herz et al. 2014; Alabert et al. 2015; Hammond et al. 2017). Additionally, histone modifying enzymes use the posttranslational modifications present on these recycled tetramers to appropriately modify the new H3–H4 tetramers that become incorporated nearby (Fig. 2B; Ayyanathan et al. 2003; Hansen et al. 2008; Margueron et al. 2009; Alabert and Groth 2012; Alabert et al. 2015; Audergon et al. 2015; Ragunathan et al. 2015). Third, the conservative model of histone recycling suggests that preexisting H3–H4 tetramers can be biased to incorporate nonrandomly into either the leading or the lagging strand (Seale 1976; Weintraub 1976; Leffak et al. 1977; Riley and Weintraub 1979; Seidman et al. 1979; Roufa and Marchionni 1982). This mechanism could provide one daughter strand with the same epigenetic information as that in the mother cell, whereas the other daughter strand could predominantly incorporate new, unmarked histones devoid of such epigenetic information (Fig. 2C).

Strong evidence supports both the dispersive model and the conservative model of histone recycling during DNA replication. Of note, these studies have been done in various organisms and cell types, as well as in vitro. It is important to consider that histone recycling may be dif-

Figure 2. Models of histone recycling and the epigenetic effect of each. (A) Semiconservative model. The semiconservative model suggests that the H3–H4 tetramer is split into two dimers that are evenly distributed between the two new double-stranded DNA daughters. Although it is a potentially elegant solution to evenly distributing relevant epigenetic information between the two new daughter strands, it has been shown that the H3–H4 tetramer rarely, if ever, splits and the tails of H3 and H4 within each tetramer are not symmetrically modified (Xu et al. 2010; Chen et al. 2011; van Rossum et al. 2012; Voigt et al. 2012). Thus, the epigenetic information of that previously unreplicated region would not be preserved equally onto the two daughter strands. (B) Dispersive model. The dispersive model of histone recycling proposes that the H3–H4 tetramer remains intact, but the tetramers and dimers disassemble ahead of the fork and are still randomly distributed onto the leading and lagging strands behind the fork (Jackson and Chalkley 1981, 1985; Alabert and Groth 2012; Herz et al. 2014; Alabert et al. 2015; Hammond et al. 2017). Additionally, histone modifying enzymes use the posttranslational modifications present on these recycled tetramers to appropriately modify the new H3–H4 tetramers that become incorporated nearby and the epigenome of the mother strand is retained in both daughter strands (Ayyanathan et al. 2003; Hansen et al. 2008; Margueron et al. 2009; Alabert and Groth 2012; Alabert et al. 2015; Audergon et al. 2015; Ragunathan et al. 2015). (C) Conservative model. The conservative model of histone recycling suggests that preexisting H3–H4 tetramers can be biased to incorporate nonrandomly into either the leading or the lagging strand (Seale 1976; Weintraub 1976; Leffak et al. 1977; Riley and Weintraub 1979; Seidman et al. 1979; Roufa and Marchionni 1982). Such a mechanism could provide one daughter strand with the same epigenetic information as that in the mother cell, whereas the other daughter strand predominantly incorporates new, unmarked histones devoid of such epigenetic information.

ferent during DNA replication depending on the biological context. For example, how stem cells maintain their stemness through many rounds of mitosis has been a long-standing question in the epigenetics field. Our finding that preexisting histones are selectively retained in the renewed stem cell daughter, whereas newly synthesized histones are enriched in the differentiating daughter cell in *Drosophila* male germline stem cells (GSCs) suggests that the predominant mechanism of histone recycling may be the conservative model (Tran et al. 2012; Tran et al. 2013; Xie et al. 2015; Snedeker et al. 2017; Xie et al. 2017). Our finding also indicates that the asymmetric epigenome established during DNA replication needs to be recognized and properly segregated by potentially polar-

ized mitotic machinery. Next, we will discuss how chromatin-bound *cis*-factors and non-chromatin-bound *trans*-regulators coordinate to ensure nonrandom sister chromatid segregation.

THE CENTROMERE: AN EPIGENETIC BASIS TO DISTINGUISH ASYMMETRIC SISTER CHROMATIDS

Centromeres direct chromosome segregation during mitosis, which is mediated by the recruitment of the kinetochore as well as microtubules. Centromeres are epigenetically defined in most eukaryotes by a centromere-

Figure 3. The “centromere drive” hypothesis holds that centromere strength directs chromosome segregation in meiosis I (MI). When two telocentric chromosomes fuse in a natural population to create one metacentric chromosome, (A) if telocentric chromosome fusion creates a metacentric chromosome with a stronger centromere, then the metacentric chromosomes preferentially segregate to the egg in MI, or (B) if telocentric chromosome fusion creates a metacentric chromosome with a weaker centromere, then 40% of the metacentric chromosomes segregate to the polar body in MI (Chmátal et al. 2014).

specific histone H3 variant known as the centromere identifier in flies and CENP-A in mammals (Palmer et al. 1987; Allshire and Karpen 2008). The centromeric histones have undergone rapid evolution and the length of DNA defined as the centromeric region has greatly increased through a positive selection process termed “centromere drive” (Henikoff et al. 2001; Henikoff and Malik 2002; Malik 2009). An expansion of the centromeric DNA by recombination could create a centromere that has increased microtubule binding ability, which could, in turn, lead to preferential chromosome transmission, such as that found during female meiosis. For example, the mouse karyotype typically consists of $2n = 40$ telocentric chromosomes, but numerous natural populations show dramatically reduced chromosome numbers in which $2n = 22$ chromosomes, a phenomenon attributed to Robertsonian (Rb) fusion, a chromosomal rearrangement that joins two telocentric chromosomes to create one metacentric chromosome (White et al. 2010). Retention of a metacentric chromosome in offspring depends on the direction of chromosome segregation during meiosis I (MI). The direction of chromosome segregation depends on centromere strength; stronger centromeres have more CENP-A protein and outer kinetochore components and, hence, higher microtubule binding ability. Therefore, the stronger centromeres are preferentially retained in the egg, whereas the weaker centromeres are preferentially segregated in the polar body during meiosis (Fig. 3; Chmátal et al. 2014). A similar event has also been reported in the budding yeast *Saccharomyces cerevisiae*, in which the inner and outer kinetochore components show asymmetric segregation in a lineage-specific manner during meiosis (Thorpe et al. 2009).

However, this phenomenon has not been reported during mitosis. Therefore, it would be worth testing a hypothesis similar to “centromere drive” during ACD. Because two distinct daughter cells arise from ACD, it is plausible that epigenetic asymmetry on sister chromatids would in-

clude sister centromeres. This would allow the mitotic machinery to distinguish the sister chromatids.

CHROMATIN ORGANIZATION: A MECHANISM FOR *TRANS*-NUCLEAR MEMBRANE COMMUNICATION

Chromosomes contain euchromatic and heterochromatic domains, which have distinct nuclear functions and organization throughout development (Misteli 2007; Mekhail and Moazed 2010; Rajapakse and Groudine 2011). The most striking examples of chromatin organization are the centromere cluster (Rabl configuration), heterochromatin cluster (chromocenter), and telomere cluster (bouquet configuration) at the nuclear periphery (Funabiki et al. 1993; Jin et al. 1998; Zickler and Kleckner 1998; Scherthan and Schönborn 2001; Fransz et al. 2002; Guenatri et al. 2004; Fang and Spector 2005; Zickler 2006). The centromere cluster and the pericentromeric heterochromatin region could provide a location where specific factors are concentrated to facilitate communication between chromosomes and microtubules. For example, kinetochore proteins and heterochromatin factors, such as HP1 and H3K9me2/3, could concentrate at the centromere or pericentromeric regions (Bernard et al. 2001; Kawashima et al. 2007). Interestingly, mutations of kinetochore components Mis6 and Nuf2 (NDC80 complex) result in centromere declustering (Appelgren et al. 2003; Asakawa et al. 2005). Nevertheless, how kinetochore components are linked to the nuclear envelope and mediate centromere dynamics remains elusive.

Mitotic hallmarks, such as phosphorylation of key histone residues including H3S10P, H3T3/T6P, H3.1/2S28P, and H1.4S26P, are shown to be predominantly associated with old histones at early mitosis in cultured human cell lines (Lin et al. 2016). We wanted to define the mechanism

Figure 4. Pericentromeric heterochromatin modifications could regulate centromere and microtubule interactions, as well as nonrandom sister chromatid segregation. (A) Sequential phosphorylation of T3 on old histone H3 before new H3 at the pericentromeric region during mitosis in *Drosophila* male GSCs ensures nonrandom sister chromatid segregation. (B) Expression of H3T3A, where T3 of histone H3 is mutated to alanine (A), randomizes the segregation pattern of sister chromatids (Xie et al. 2015, 2017).

(s) by which sister chromatids might be recognized and segregated in an asymmetric manner. To begin to address this question, we recently reported that the H3T3P mark at pericentromeric regions distinguishes old and new histones in *Drosophila* male GSCs (Fig. 4A; Xie et al. 2015). Furthermore, misregulation of this phosphorylation leads to randomized inheritance of old and new H3, as well as both GSC loss and progenitor germ cell tumor phenotypes. This suggests that asymmetric phosphorylation of H3T3 at the pericentromeric regions may be one mechanism by which the mitotic machinery can recognize and faithfully segregate asymmetric sister chromatids (Fig. 4B).

CENTROSOMES AND MICROTUBULES: MECHANICAL TOOLS FOR NONRANDOM SISTER CHROMATID SEGREGATION

The centrosome is a complex molecular structure that functions as the major microtubule-organizing center in the cell. Recent studies have revealed intriguing asymmetry between mother and daughter centrosomes, as well as the involvement of such asymmetry in a number of critical cellular processes. Two centrosomes are distinct from each other, partly resulting from their microtubule nucleation activity and their age differences. Interestingly, the older of

Figure 5. Nonrandom segregation of sister chromatids, asymmetric centrosome inheritance, and asymmetric histone inheritance during *Drosophila* male germline stem cell (GSC) asymmetric cell division (ACD). Asymmetric GSC divisions give rise to two daughter cells: a self-renewed GSC that remains in proximity to the niche (blue hub cells), and a differentiating daughter cell that migrates to the distal side of the cell, resulting from a perpendicular spindle orientation relative to the niche. During this division, mother (green) and daughter (orange) centrosomes are asymmetrically inherited by the self-renewed GSC and the differentiating daughter cell, respectively. Non-random sister chromatid segregation of the X/Y sex chromosomes was shown in male GSCs using CO-FISH (chromosome-oriented fluorescence in situ hybridization) in combination with strand-specific probes to distinguish sister chromatids. Sex chromosomes (purple and blue outlined chromatids) show an ~85:15 bias segregation during male GSC cell division (Yadlapalli and Yamashita 2013). A dual-color labeling strategy to distinguish preexisting (green) versus newly synthesized (red) canonical histone H3 revealed that old histone H3 is selectively retained in the self-renewed GSC (green nuclei), whereas newly synthesized H3 is enriched in the differentiating daughter cell (red nuclei) (Tran et al. 2012).

the two centrosomes has been shown to nucleate microtubules considerably earlier, which is correlated with a differential response to signaling molecules (Fig. 5; Rebollo et al. 2007; Rusan and Peifer 2007; Anderson and Stearns 2009; Pelletier and Yamashita 2012). The observation that centrosome age could be associated with differential response to various signaling cues has raised the possibility that the inherent asymmetry of centrosomes could contribute to the determination of distinct cell fates during ACD.

In the fission yeast *Schizosaccharomyces pombe* during meiosis, LINC (linker of nucleoskeleton and cytoskeleton) connects the centrosome with telomeres (Tomita and Cooper 2007), whereas during mitosis LINC connects the centrosome with centromere, rather than telomeres (Fernández-Álvarez et al. 2016). Loss of such contacts during meiosis or mitosis abolishes normal spindle formation (Tomita et al. 2013; Fennell et al. 2015), suggesting that the *trans*-nuclear envelope contacts through LINC play an important role in mediating cross talk between centrosomes and chromosomes, residing in the cytoplasm and the nucleus, respectively.

Studies have revealed that the *Drosophila* male GSCs and mouse neural glial progenitor cells inherit the mother centrosome (Yamashita et al. 2007; Wang et al. 2009), whereas *Drosophila* neuroblasts and female GSCs inherit the daughter centrosome (Conduit and Raff 2010; Januschke et al. 2011). This difference in centrosome inheritance patterns during ACD provokes the speculation that the developmentally programmed centrosome located at the stem cell side, whether mother or daughter, might bear fate determinants or other characteristics that contribute to stem cell fate. Therefore, it is conceivable that early

microtubule nucleation at one of the two centrosomes, either age-dependent or in differential response to signaling molecules at the stem cell side, could engage in cross talk with centromeric chromatin through LINC to ensure preferential sister chromatid attachment.

In summary, a cohort of *trans* factors, such as centrosomes, microtubules, nuclear membrane, and kinetochore complex, and *cis* factors, such as centromeres and epigenetic modifications on chromatin, act together in differential recognition and nonrandom segregation of sister chromatids. In the future, more studies are needed to understand how this axis of asymmetry, centrosome–microtubule–nuclear membrane kinetochore–centromere–chromatid, is regulated and whether disruption of this axis leads to any cellular defects.

ASYMMETRIC INHERITANCE OF CELL FATE DETERMINANTS IN UNICELLULAR ORGANISMS

Over the past three decades, the mating type switching behavior in two eukaryotic yeasts, the budding yeast *S. cerevisiae* and the fission yeast *S. pombe*, has served as an exceptional model to dissect the mechanisms of asymmetric cell fate specification (Klar 2007). Mating type switching is accomplished by two functionally similar but molecularly distinct processes in *S. cerevisiae* and *S. pombe*. The genomes of these species encode a three-cassette gene structure containing one active and two silent copies of the mating type locus. These cells can alter their mating type through a programmed DNA rearrangement process and execute it through the cleavage of the

active locus, whereas a copy of a silent locus serves as a donor for synthesis-dependent strand annealing (Dalgaard and Klar 2001). In *S. pombe*, the mating type switching pattern results from the inheritance of a specific parental DNA strand, which is dependent on a strand-specific epigenetic imprint that occurs during lagging-strand DNA synthesis (Dalgaard and Klar 2001). Generation of this imprinting phenomenon is dependent on the orientation of DNA replication at the active mating type locus, *mat1* (Dalgaard and Klar 1999). Specifically, it is thought that one or two ribonucleotides form the imprint and that these RNA residues may have been originally used to prime DNA synthesis on the lagging strand, which may be ligated and not removed by the DNA repair machinery during the first S phase (Vengrova and Dalgaard 2006). This imprint is then maintained until the next S phase, when the leading-strand replication complex is stalled at the imprint locus (Fig. 6; Vengrova and Dalgaard 2004). This stalled fork induces a recombination event between *mat1* and one of the two silent donor cassettes, *mat2P* or *mat2M*, leading to mating type switching. As a result of this mechanism, one of the two daughters of a newly switched cell inherits a switch in mating locus, and one of the four granddaughters has the switched mating type. Together, these findings show that asymmetries inherent in DNA replication can be developmentally regulated to ensure distinct cell fate determination after cell division. Considering that *S. pombe* is a haploid organism, it does not require selective segregation of sister chromatids; instead, the daughter cell inherits the cell fate epigenetic determinant from the chromosome randomly from the parental cell. For such mechanism to function reliably in a diploid organism, selective recognition of the distinct chromatid would be necessary.

Mating type gene switching in *S. cerevisiae* is mediated by an enzyme not found in *S. pombe*, the HO endonuclease, which is responsible for the programmed creation of a site-specific double-strand break at the active mating type locus, *MAT* (Haber 2012). Mating type DNA rearrangements occur exclusively in mother cells, not in the daughter cells or spores. The discovery of the exclusive expression of HO in the mother cells led to another breakthrough in asymmetric inheritance of cell fate determinants (Bobola et al. 1996; Sil and Herskowitz 1996; Long et al. 1997). *S. cerevisiae* achieves mitotic proliferation through a highly polarized ACD, giving rise to a smaller daughter (bud) cell. After each ACD, the cell-fate determinant *asymmetric synthesis of HO* (*Ash1*) mRNA, encoding a HO-specific transcriptional repressor, is asymmetrically inherited by the bud cell (Long et al. 1997). This asymmetric localization is mediated by a ribonucleoprotein complex, which is transported across the actin cytoskeleton to the distal tip where translation of *Ash1* mRNA occurs (Cosma 2004). To date, more than 20 mRNAs have been found to be asymmetrically inherited during *S. cerevisiae* cell division (Shepard et al. 2003; Jambhekar et al. 2005).

Cell polarity and spindle orientation are coordinated before mitosis and mediated by three polarized cytoskeletal systems, including actin, septins, and microtubules (Bi and Park 2012). Orientation of the yeast spindle pole

Figure 6. Mating type switching in *Schizosaccharomyces pombe*. (A) Arrangement of the mating type region, displaying switchable *mat1* and silent donor loci *mat2P* and *mat3M*. The *mat1* locus contains either plus (green coding) or negative (purple coding) regions derived from the donor cassettes, *mat2P* (plus information) or *mat3M* (minus information). Homology domains H1, H2, and H3 are annotated. Arrows indicate recombination events leading to mating type switching. SAS1 and SAS2 are *cis*-acting sequences involved in imprinting. (B) Generation of the imprint is regulated by the direction of *mat1* DNA replication. The imprint (shown as a red star) is installed if the specific strand is the lagging strand (designated by the dashed red line). During the next S phase, when the leading-strand replication complex (designated by the blue arrow) encounters the imprint, a double-strand break (DSB) occurs. The broken strand then uses one of the two donor loci, either *mat2P* or *mat3M*, as a template to repair the *mat1* locus.

body (SPB), the equivalent of the centrosome, is linked to a stereotypic pattern of SPB inheritance (Pereira and Yamashita 2011). Spindle formation starts in the mother cell body with the older centrosome oriented toward the bud, which establishes spindle polarity, directing orientation of the mitotic spindle along the mother–bud axis and the inheritance of the old SPB by the daughter cell. A similar phenomenon is observed in the ACDs of the *Drosophila* male GSCs, as well as mouse neural glial progenitor cells, where the mother centrosome is preferentially retained near the hub–GSC interface and by the radial glial progenitors that remain in the ventricular zone, respectively (Yamashita et al. 2007; Wang et al. 2009). The molecular mechanisms that govern the establishment of this cell polarity and spindle orientation have been highly conserved throughout evolution (Pereira and Yamashita 2011). This role in establishing polarity, as well as preferential asymmetric inheritance, raises the intriguing possibility that

distinct centrosomes may be associated with recognizing and segregating cell fate determinants, such as individual chromatids, which has been previously suggested for adult stem cells, including *Drosophila* male GSCs and mouse skeletal muscle satellite cells (Shinin et al. 2006; Xie et al. 2015).

ACD IN *DROSOPHILA*—A MODEL OF NONRANDOM SEGREGATION OF SISTER CHROMATIDS AND ASYMMETRIC EPIGENETIC INHERITANCE

Nonrandom segregation of sister chromatids occurring in the germline was uncovered by using chromosome-oriented fluorescence in situ hybridization (CO-FISH) to resolve individual chromatid inheritance (Yadlapalli and Yamashita 2013). The CO-FISH method revealed that sex chromosomes (X and Y) show an ~85:15 strand bias during male GSC ACD (e.g., 85% of GSCs inherit the Watson strand [W] and 15% of GSCs inherit the Crick strand [C] at each GSC division). Autosomes including both the second and the third chromosomes, however, display a random segregation pattern (50:50), but show a consistent co-segregation mode (i.e., WW:CC instead of WC:CW) (Fig. 5). Of note, earlier studies using BrdU to test global DNA segregation showed that male GSCs do not follow the immortal strand model (Yadlapalli and Yamashita 2013). These results, combined with our work showing that H3T3P distinguishes old versus new his-

tones in dividing GSCs, suggest that epigenetic differences that distinguish sister chromatids might coordinate selective chromatid segregation and direct cell fate after mitosis. Together, these findings reveal the presence of asymmetric epigenetic inheritance during cell division, which may maintain GSC cell identity, while concomitantly resetting the chromatin structure in the differentiating daughter cell to ensure proper cell fate specification.

NONRANDOM SEGREGATION OF DNA STRANDS IN MAMMALIAN CELLS AND DISEASE

Adult skeletal muscle in mammals has an extraordinary regenerative capacity after injury. The regenerative precursor cells originate from the satellite muscle cells, a tissue-specific adult stem cell population (Collins et al. 2005). Mononucleated satellite cells are mitotically quiescent and reside in a niche under the basal lamina, or basement membrane, juxtaposed to the muscle fiber. As skeletal muscle stem cells, satellite cells divide asymmetrically to maintain the stem cell population and differentiate, leading to new myofiber formation (Fig. 7; Kuang et al. 2007). Interestingly, the orientation of the cell division within the satellite cell niche determines cell fate (Kuang et al. 2007). Sister cells arising from a satellite cell division are found in either a planar orientation where both cells remain in direct contact with the basal lamina and myofiber or in an apical-basal orientation where one

Figure 7. Orientation of muscle satellite cell division determines cell fate. (A) Satellite cells (blue) reside under the basal lamina adjacent to the myofibril (red). (B) Satellite cells enter either a symmetric cell division or an ACD. ACD (i.e., when the mitotic spindle is oriented perpendicular to the muscle fiber) generates a self-renewing (expressing Pax7+/Myf5-, blue cell) and a differentiating daughter cell (Pax7+/Myf5+, red cell). Symmetric divisions (i.e., when the mitotic spindle is oriented parallel to the muscle fiber) generate two self-renewing cells that are both Pax7+/Myf5-. (C) Co-segregation of template DNA strands labeled with BrdU (purple) and asymmetric distribution of Numb (green) to one daughter cell were observed in asymmetrically dividing myoblasts derived from satellite cells during anaphase in an 11:1 bias.

daughter cell is pushed toward the basal lamina and the other is oriented apically toward the myofiber, occurring with 92% and 8% frequency, respectively. Further studies revealed that apical–basal orientation of cell divisions resulted in asymmetric cell fates. The daughter cell attached to the basal lamina remained a stem cell, whereas the daughter cell that loses contact with the basal lamina becomes a committed myogenic cell. In contrast, stem cell divisions in a planar orientation were symmetric and generated identical daughter cells.

Satellite cells express the transcription factor Pax7, but not Myf5 (Pax7⁺/Myf5[−]), whereas the asymmetric differentiating daughter cell expresses both Pax7 and Myf5 (Pax7⁺/Myf5⁺) (Kuang et al. 2007). Established experimental approaches to track segregation of old versus nascent DNA involve single or consecutive rounds of halogenated nucleotide analog labels. The thymidine analog, 5-bromo-2′-deoxyuridine (BrdU), can be used to label newly synthesized DNA strands in freshly isolated satellite cells from single fibers. Three days after labeling, single parental cells generated two daughter cells, one BrdU⁺ and another BrdU[−], indicating that template DNA strands can be co-segregated in adult muscle stem cells at a 7% frequency (Shinin et al. 2006). Furthermore, template DNA strands were found to co-segregate with the asymmetric cell fate determinant Numb (Fig. 7C). The low frequency observed may be an underestimate owing to the *ex vivo* culture conditions, but it could be higher if tested in an *in vivo* tissue context or if investigated to specifically determine DNA inheritance in asymmetrically dividing Pax7⁺/Myf5[−] cells. Indeed, later experiments observed an increased frequency (38%) of asymmetric template DNA inheritance in Pax7⁺/Myf5[−] cells (Kuang et al. 2007).

During myogenic lineage commitment, satellite cells differentiate and express Desmin, a muscle-specific intermediate filament. In combination with Sca-1 (stem cell antigen-1 protein, a marker for undifferentiated muscle progenitors), cell pairs were examined to determine whether specific templates segregated with specific cell fates. Strikingly, 79% of pairs showed Desmin expression only in the daughter inheriting nascent BrdU⁺ templates (Conboy et al. 2007). Among pairs whose templates were labeled by BrdU as symmetrically inherited, nearly all were symmetric for Desmin expression. Furthermore, 84% of asymmetric Desmin-positive cells showed asymmetry of Sca-1, demonstrating that older templates co-segregate with the less differentiated cells. An independent study using CO-FISH with single-chromatid resolution showed that asymmetric DNA segregation includes all chromosomes. Based on relative Pax7 levels, a population of high Pax7-expressing satellite cells was characterized to perform template strand co-segregation at a higher frequency (Rocheteau et al. 2012). Together, these experiments provide the evidence of template strand co-segregation based on template age, demonstrating that asymmetric co-segregation is associated with cell fate determination. It remains to be elucidated (1) how template strand age is monitored and recognized during cell lineage progression and (2) whether this co-segregation of tem-

plate DNA is linked to gene regulation or silencing of specific loci in the satellite cells.

Cardiac resident stem cells in neonatal and adult mammalian hearts have been identified by distinct membrane markers and transcription factors, including c-kit and Nkx2.5, respectively (Beltrami et al. 2003). These c-kit-positive endogenous cardiac stem cells (eCSC) are self-renewing, are multipotent, and can divide through ACD (Beltrami et al. 2003; Urbanek et al. 2006). Furthermore, these eCSCs have been shown to be necessary and sufficient for myocyte regeneration, leading to anatomical and functional myocardial recovery following myocardial damage (Ellison et al. 2013). The c-kit-positive CSCs were isolated and tested for asymmetric chromatid segregation using the thymidine analogs BrdU and IdU in combination with different pulse-chase time points to detect old versus nascent DNA strands (Kajstura et al. 2012; Sundararaman et al. 2012). From 4% to 7% of c-kit-positive CSCs isolated from myocardial samples displayed asymmetric inheritance of nascent DNA detected during anaphase and telophase in two independent studies (Kajstura et al. 2012; Sundararaman et al. 2012). This range significantly exceeds the probability that a random segregation of chromatids would yield an asymmetrical distribution of labeled nucleotides. Therefore, further characterization is necessary to determine whether a subpopulation of c-kit-positive CSCs exists and, similar to muscle satellite cells, shows increased ACD and nonrandom chromatid segregation. CO-FISH experiments using chromosome-specific probes could address individual chromosome inheritance upon ACD.

Recent examples have also shown chromatid-biased DNA segregation in colon crypt cells (Falconer et al. 2010). To identify sister chromatids, CO-FISH with unidirectional probes specific for centromere and telomere repeats were used in combination with BrdU to label nascent chromosomes. Mice were injected with BrdU hourly for 12 h to label actively dividing cells; colon tissue was then fixed, sectioned, and subjected to CO-FISH probes. Sister nuclei showing reciprocal, asymmetric CO-FISH fluorescence were found throughout the colon crypt, indicating that sister chromatids of most chromosomes were segregating nonrandomly. However, the asymmetry was observed for only a subset of the sister chromatids in any cell pair within the colon crypt. This reflects a possibility that a subset of colon cells selectively segregates sister chromatids from most, but not all, chromosomes. Whether specific chromatids are selectively captured within these cells remains to be investigated.

To date, several studies have provided evidence for nonrandom DNA segregation in diverse cell types. It is reasonable to ask if this phenomenon is widespread. An earlier study of chromosome strand segregation based on site-specific recombination markers in mouse embryonic stem cells revealed a nonrandom distribution of chromosome 7 (Armakolas and Klar 2006). However, two recent studies using CO-FISH indicate that this is not the case and that chromosomes are randomly segregated (Falconer et al. 2012; Sauer et al. 2013). Studies using BrdU to follow the ancestral DNA during the first ACD of the

Caenorhabditis elegans embryo also failed to detect asymmetric segregation of DNA (Ito and McGhee 1987). Furthermore, examples of adult stem cells that do not asymmetrically segregate chromosomes include hair follicle stem cells (Sotiropoulou et al. 2008) and hematopoietic stem cells (Kiel et al. 2007). Together, studies so far indicate that asymmetric segregation of DNA strands occurs in some, but not all, stem cell types.

Many types of adult stem cells undergo ACD to balance self-renewal and differentiation for normal tissue homeostasis. Misregulation of any of the molecular mechanisms that control the asymmetric segregation of cell fate determinants during stem cell divisions may result in hyperproliferation of the stem cell compartment, leading to tumorigenesis, or a loss of the stem cell population, resulting in tissue dystrophy (Knoblich 2010). Previous studies suggest that tumors contain rare cell populations that have stem cell properties, and when injected into immunocompromised mice, they are able to self-renew and generate heterogeneous tumors (Cho and Clarke 2008; Vermeulen et al. 2008; Charafe-Jauffret et al. 2009). These studies indicate that a subpopulation of tumor cells can self-renew and repopulate the heterogeneous tumor, suggesting tumor cell repopulation may occur via ACD within subpopulations of tumor cells. Recent studies in both primary lung cancer cells and cell lines indicate a subset of cells that divide asymmetrically, segregating their template DNA strands exclusively to one daughter cell (Pine et al. 2010). Specifically, double-label experiments using IdU and CldU (chlorodeoxyuridine) in combination with real-time imaging in non-small cell lung cancer (NSCLC) cells showed that old template strand DNA segregated asymmetrically in anaphase and telophase cells. Of the seven NSCLC cell lines examined, asymmetric segregation of template DNA ranging from 0.5% to 6.8% was observed. Furthermore, primary NSCLC tumors displayed an enriched population of cells that asymmetrically segregated template DNA, ranging from 12.5% to 18%, which could reflect an increased concentration of asymmetrically dividing cells within the primary tumor or upon ex vivo expansion. Segregation of the template DNA strands correlated significantly with distinct cell fate markers, including co-segregation with cell fate marker CD113, labeling a tumor subpopulation that could repopulate the entire cell population of lung tumor cells in vitro and in vivo (Eramo et al. 2008; Bertolini et al. 2009). Although these studies have uncovered a significant population of lung cancer primary cells and cell lines able to coordinate asymmetric segregation of template DNA, our understanding of the cell fate choices influenced by this asymmetry is limited and awaits further investigation.

CONCLUSION

One of the greatest discoveries in the 20th century was the double helix structure and semiconservative duplicating process of DNA, providing an elegant and fundamental principle of life. However, as discussed here and reviewed in Snedeker et al. (2017), the inherent asymme-

try of DNA replication and the increasing knowledge about the polarity in mitosis raise some questions. Further research will explore whether symmetric outcomes arise from tightly regulated asymmetric molecular and cellular processes, or whether symmetry is the default pathway and is then broken by asymmetric processes.

In reality, both symmetric and asymmetric outcomes are required to build up a multicellular organism originating from a single cell, a fertilized egg, to produce an individual human being made up of hundreds of cell types. Even though most cells in our bodies carry identical DNA sequences, only a subset of these sequences turn on expression at the proper time, in the right place, and with the precise level during development and homeostasis. It is well recognized that the distinct epigenetic information contained in each cell type defines its unique gene expression program. However, how the epigenetic information contained in the parental cell can be maintained, or changed, in the daughter cells remains largely unknown. This question is extremely difficult to address because the epigenome is composed of numerous components that dynamically change their composition. Nonetheless, this question is central to our understanding of the fundamental principles of biology and our ability to develop new treatments against human diseases including birth defects, neurodegenerative disease, tissue dystrophy, infertility, and cancers. Asymmetric histone inheritance could represent the mechanism that maintains equilibrium between the rigidity of genetic information and the plasticity of epigenetic information. We anticipate that future work will address whether this mechanism is used at specific gene loci for differential gene expression upon ACD and whether this mechanism is also applicable to other cell types or in other organisms.

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