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The Inherent Asymmetry of DNA Replication

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Keywords

DNA replication, asymmetric, histones, epigenetic inheritance, chromatin

Abstract

Semiconservative DNA replication has provided an elegant solution to the fundamental problem of how life is able to proliferate in a way that allows cells, organisms, and populations to survive and replicate many times over. Somewhat lost, however, in our admiration for this mechanism is an appreciation for the asymmetries that occur in the process of DNA replication. As we discuss in this review, these asymmetries arise as a consequence of the structure of the DNA molecule and the enzymatic mechanism of DNA synthesis. Increasing evidence suggests that asymmetries in DNA replication are able to play a central role in the processes of adaptation and evolution by shaping the mutagenic landscape of cells. Additionally, in eukaryotes, recent work has demonstrated that the inherent asymmetries in DNA replication may play an important role in the process of chromatin replication. As chromatin plays an essential role in defining cell identity, asymmetries generated during the process of DNA replication may play critical roles in cell fate decisions related to patterning and development.

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1. INTRODUCTION: A HISTORY OF DNA REPLICATION

The combined efforts of Rosalind Franklin, Francis Crick, James Watson, and Maurice Wilkins provided the scientific community at large with an understanding of how two antiparallel strands of DNA are able to pair together in the double-helix structure (Avery et al. 1944; Franklin & Gosling 1953a,b; Watson & Crick 1953a,b; Wilkins et al. 1953). Following this groundbreaking discovery, those in the field of DNA research sought to understand the mechanisms by which DNA is able to copy itself to allow for transmission of genetic information from parents to subsequent generations. Indeed, at the time of this discovery, the structure of the double helix itself seemed to suggest an elegant copying mechanism by which both strands, once separated, could simultaneously serve as templates for the copying of duplicate strands. Subsequent experiments by Meselson & Stahl (1958) further demonstrated that this model of DNA replication was indeed the primary method utilized by cells to replicate their genomic material. This model, termed semiconservative DNA replication, suggests that both existing strands are used as templates from which two identical strands of DNA, termed sister chromatids, can be produced and eventually segregated during mitosis.

However, the two strands that result from semiconservative DNA replication are not always precise duplicates of one another. Asymmetries between sister chromatids arising from the process of DNA replication can, in large part, be understood by revisiting the basic physical structure of the DNA molecule. The antiparallel structure of the DNA double helix is extremely important in the context of DNA replication, specifically because of the enzymatic activity of the molecules responsible for new DNA synthesis. At the start of eukaryotic DNA replication, the double-stranded DNA (dsDNA) molecule separates into two single-stranded molecules at discrete locations along the length the chromosome, forming structures known as replication bubbles. At the two edges of the replication bubble sits the replication fork, a structure defined as the junction between

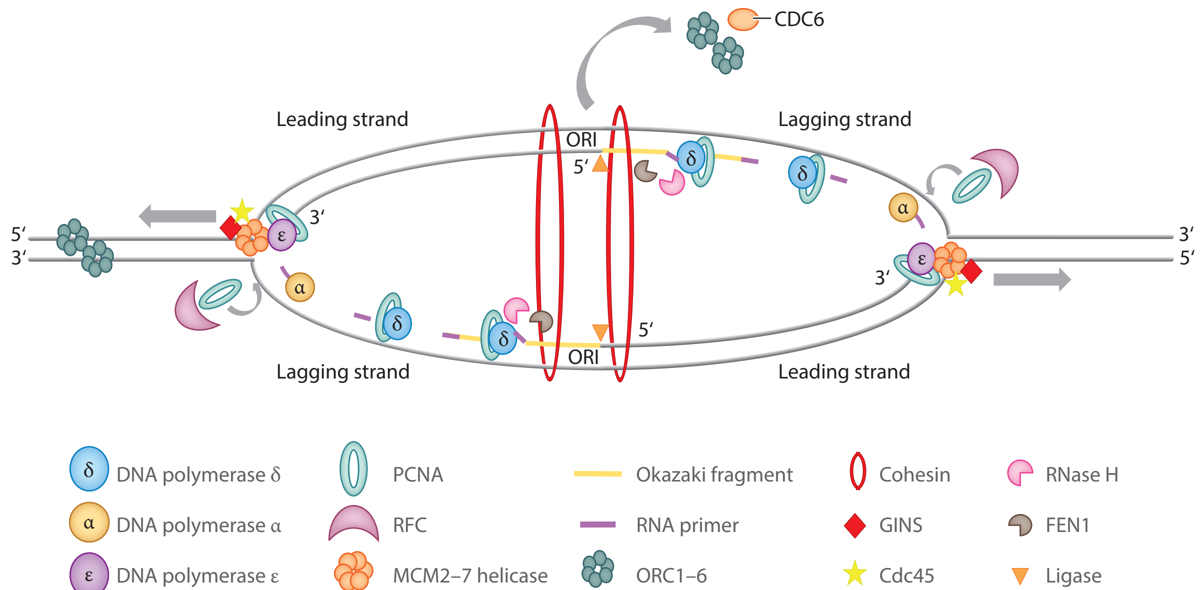


Figure 1

Overview of DNA replication. The fork fires as the MCM2–7 helicase proceeds out in a bidirectional fashion from the origin of replication (ORI), unwinding double-stranded DNA as it goes. ORC proteins and Cdc6 dissociate from the origin, where they were initially bound to facilitate helicase loading. CDC45 and GINS travel with the MCM2–7 helicase to create the CMG replication complex. The leading strand is synthesized primarily through the actions of DNA polymerase ε. The lagging strand is discontinuously synthesized primarily through the actions of DNA polymerases α and δ. Polymerase α synthesizes the RNA primer needed to initiate DNA synthesis. The lagging strand is additionally processed by FEN1 and ligase to complete synthesis. The RFC complex is responsible for loading PCNA, which acts as a processivity factor for polymerases ε and δ. Cohesins link the two sister chromatids following passage of the replication fork.

dsDNA and newly separated single-stranded DNA (ssDNA) (**Figure 1**). Following separation of the DNA duplex, each of the two newly separated ssDNA molecules serves as a template to direct the synthesis of new DNA. As new DNA is synthesized, replication forks move outward in opposite directions to generate new DNA templates by separating additional parental duplex DNA in a process termed replication fork progression. The molecules responsible for reading these newly exposed ssDNA templates are known as DNA polymerases and were first discovered in the laboratory of Arthur Kornberg. Kornberg and his colleagues found that DNA polymerases can elongate DNA from a ssDNA template and a primer by adding new bases specifically in the 5′-to-3′ direction. This directional specificity (5′-to-3′ addition of new bases) is due to the fact that DNA polymerase requires a free 3′ hydroxyl (–OH) group on the growing end of the newly synthesized DNA strand to catalyze the formation of phosphodiester bonds during the process of strand elongation. Because DNA polymerase can synthesize new DNA only in the 5′-to-3′ direction, ssDNA template strands must be read in a 3′-to-5′ direction to preserve the antiparallel structure of the parental DNA duplex. Furthermore, an important consequence of the antiparallel structure of the parental DNA duplex is that, during the process of replication fork progression, the two template strands are opened in opposite directions: one in the 3′-to-5′ direction and the other in the 5′-to-3′ direction (**Figure 1**). As DNA polymerase can read template DNA only in the 3′-to-5′ direction, only the strand whose 5′ end is oriented in the same direction as replication fork progression can be read continuously. As a result, only the strand synthesized from the

template strand with its 5′ end oriented in the same direction as replication fork progression—termed the leading strand—will be able to be synthesized in a continuous fashion (Bessman et al. 1956, 1958; Kornberg 1989; Lehman et al. 1958). Conversely, the lagging strand, which is synthesized from the template strand oriented with its 5′ end opposite to the direction of fork progression, is synthesized in short, discontinuous segments. These segments, termed Okazaki fragments after their discoverers, are synthesized in the opposite direction of fork movement and are typically sized between 100 and 200 base pairs (bp) in eukaryotes and between 1,000 and 2,000 bp in prokaryotes (Balakrishnan & Bambara 2013, Okazaki et al. 1968, Sakabe & Okazaki 1966). In summary, by virtue of the antiparallel organization of the DNA double helix in conjunction with the unidirectional enzymatic mechanism of DNA polymerase, DNA replication is inherently asymmetric. As a result, DNA synthesis occurs in two distinct mechanisms: continuous (leading-strand) synthesis and discontinuous (lagging-strand) synthesis. In this review, we elaborate on new and exciting research demonstrating the important biological function these asymmetries have in roles ranging from adaptation and evolution to cell fate decisions during development.

2. DNA REPLICATION

2.1. Initiation and Elongation of DNA Replication

Before discussing the consequences of the asymmetric processes and resulting outcomes of semiconservative DNA replication, it is useful to briefly discuss the basics of DNA replication. In doing so below, we elaborate on the fundamental reasons why the process of DNA replication is an inherently asymmetric event. Generally, in this review we discuss what is known about DNA replication and chromatin establishment in eukaryotes. When possible, we address findings in metazoans, although a large portion of DNA and chromatin replication studies have been conducted in unicellular organisms and viruses.

2.1.1. Early steps and fundamentals of DNA replication. Generally, the goal of DNA replication in dividing cells is to produce two identical sister DNA molecules from one parent DNA molecule with as high fidelity as possible without underreplicating or rereplicating any region of the genome. To propagate their genome, every species must be able to efficiently perform this fundamental process. The basic units of DNA replication are the replicon and the replisome. The replisome refers to the protein complexes used to replicate DNA, and the replicon is the stretch of DNA replicated by a single replisome. The first step of DNA replication occurs in G1 phase immediately after the separation of sister chromatids, where the origin of replication complex, specifically ORC1–6, binds DNA (Bell & Stillman 1992). In most prokaryotes and some eukaryotes, ORC binds a specific DNA sequence denoted as Ori (Fuller et al. 1984). In higher-order eukaryotes, however, a multitude of factors help specify sites of ORC binding, but there are few clear Ori sequences as in prokaryotes and lower eukaryotes (Vashee et al. 2003). However, it is becoming clear that in metazoans a wide variety of features help specify flexible, but nonrandom, origin localization (Cayrou et al. 2011).

ORC operates by recruiting Cdc6 to a subset of origins (Hateboer et al. 1998), which in turn helps recruit the MCM2–7 and Cdt1 complex (Tsakraklides & Bell 2010). MCM2–7 is the eukaryotic helicase used in DNA replication to unwind the DNA double helix (Labib et al. 2000, Nishitani et al. 2000). After MCM2–7 is loaded, the next major step in DNA replication is firing the replication forks at the G1/S transition upon signaling from Ddk and Cdk, which among other roles phosphorylate MCM (Nougarede et al. 2000). The phosphorylated MCM2–7 is bound by Cdc45,

the limiting reagent of DNA replication, along with MCM10 (Moyer et al. 2006, Wohlschlegel et al. 2002, Zou et al. 1997). GINS and DNA polymerase ε (Pol ε) are additionally loaded at this point (Muramatsu et al. 2010). The Cdc45, MCM2–7, and GINS complex forms the CMG complex, which drives the progression of the replication fork (Gambus et al. 2006). Upon further binding of additional DNA polymerases and additional DNA replication factors, the replication fork will fire and begin to move outward along the DNA.

2.1.2. Bidirectional firing and progression of the replication fork. Upon firing, a pair of CMG complexes progress bidirectionally on the 3′-to-5′ template strand—the leading strand—in eukaryotes and on the 5′-to-3′ template strand—the lagging strand—in bacteria, separating the DNA strands as the complexes progress and creating the characteristic DNA replication bubble (**Figure 1**). Behind the replication fork, one strand is opened as ssDNA in the 5′-to-3′ direction and the other strand is opened in the 3′-to-5′ direction, fundamentally because the two DNA molecules have an antiparallel orientation relative to each other (O'Donnell et al. 2013). As discussed, the antiparallel structure of the DNA molecule coupled with the enzymatic nature of DNA polymerase requires two distinct modes of DNA replication: continuous (leading-strand) synthesis and discontinuous (lagging-strand) synthesis (**Figure 1**).

2.2. Synthesis and Processing of the Leading and Lagging Strands

The leading strand is synthesized continuously in the direction of the progression of the replication fork, whereas the lagging strand is synthesized in the opposite direction of the progression of the replication fork in the form of Okazaki fragments, which must be processed and ligated to create a continuous strand. These two divergent processes underlie the asymmetry of DNA replication and are discussed in molecular detail in this section.

2.2.1. Leading-strand synthesis and essential DNA replication components. As discussed above, DNA polymerase can synthesize new DNA only in the 5′-to-3′ direction. Having to add to the 3′ end of an existing base requires that, before DNA polymerases can synthesize new DNA, an RNA primer be synthesized. In eukaryotes, this is a short 8–10-nt (nucleotide) RNA primer made by primase (Lark 1972a,b). The leading strand requires only a single RNA primer, as DNA can be continuously synthesized behind the replication fork in the 5′-to-3′ direction during leading-strand synthesis. During synthesis, DNA polymerase processivity is greatly enhanced by associating with DNA clamps. In eukaryotes, the main DNA clamp is proliferating cell nuclear antigen (PCNA), which is loaded by the clamp loader RFC behind the fork. PCNA, a trimeric complex that binds DNA, has a variety of other interacting domains that serve a host of different functions, including maintaining polymerase association with DNA while also recruiting other DNA replication factors. Such factors include FEN1, a key lagging-strand component; CAF1, a histone chaperone; and cohesin, a multiprotein complex involved in chromatin organization (Moldovan et al. 2007). Due to the variety of processes with which PCNA is associated, it is generally referred to as the tool belt of DNA replication. It has long been acknowledged that Pol ε is a leading-strand-specific polymerase (Hübscher et al. 2001). However, although it is largely accepted that Pol ε acts on the leading strand, there is a debate about whether Pol ε is the only DNA polymerase on the leading strand or whether Pol δ is a major polymerase on the leading strand, as is true on the lagging strand (Johnson et al. 2015, Pursell et al. 2007). If the latter is the case, it would stand to reason that the primary role of Pol ε would be as a proofreader on the leading strand as opposed to as a synthesizer of the bulk of DNA (Albertson et al. 2009). Both Pol δ and Pol ε have very high processivity when interacting with PCNA and have high

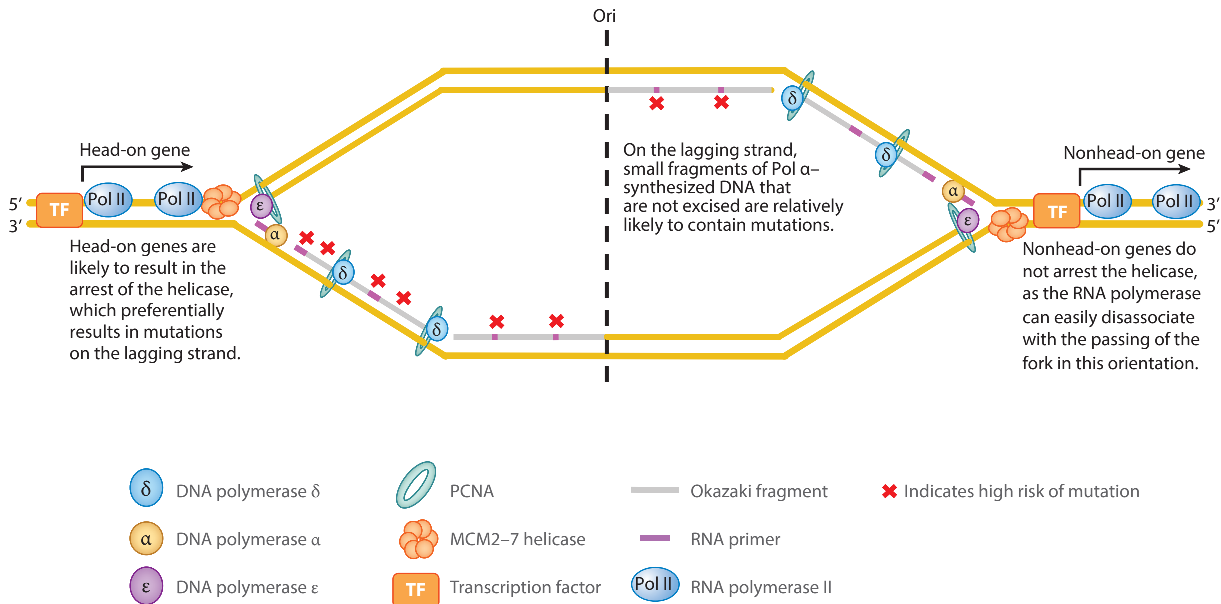


Figure 2

Mutational imbalance during DNA replication. Mutagenesis occurs asymmetrically on the two sisters during DNA replication. The lagging strand may have a higher rate of mutagenesis than the leading strand due to both incorporation by low-fidelity DNA polymerase α and the strand's increased susceptibility to mutagenesis with head-on transcription and DNA replication fork collisions.

fidelity due to their proofreading activity (Korona et al. 2011) (**Figure 2**). Therefore, regardless of the primary polymerase (Pol δ or ε) involved in leading-strand synthesis, the leading strand is synthesized continuously behind the replication fork in a highly processive and nearly error-free manner.

2.2.2. Lagging-strand synthesis. The lagging strand has a far more complicated synthesis relative to the leading strand. As the overall synthesis of the lagging strand is in the 3′-to-5′ direction, DNA polymerases working on the lagging strand must add new nucleotides in a direction opposite to that of the advancing helicase (**Figure 1**). As the polymerase is not acting directly behind the fork, the lagging-strand DNA remains single stranded for a stretch and is bound by ssDNA-binding proteins, such as RPA, which stabilize this ssDNA and recruit the Pol α/primase complex (Leonhardt et al. 2000, Ryba et al. 2010).

The lagging strand is first replicated by a Pol α/primase complex that lays down an RNA primer before synthesizing a short fragment of DNA of approximately 20 nt in length (Masai et al. 2010, O'Donnell et al. 2013). Although Pol α was the first discovered DNA polymerase and was originally thought to replicate the entire genome, it is now recognized that the primary role of Pol α is simply to start the synthesis of the lagging strand as opposed to serving as the main polymerase for the lagging strand, let alone the entire genome (Hübscher et al. 2001). Pol α has low processivity and, very importantly, far lower fidelity than the other eukaryotic polymerases (O'Donnell et al. 2013). Unsurprisingly, most of the DNA synthesized by Pol α is replaced by Pol δ, which as mentioned above is considered the main polymerase on the lagging strand. Pol δ is loaded onto the lagging strand by PCNA and RFC and, using the Pol α DNA as the primer, synthesizes the Okazaki fragments (Leonhardt et al. 2000). The length of these fragments varies

among species, but generally bacteria have relatively long fragments of approximately 1,200 nt, whereas eukaryotes have approximately 200-nt fragments whose sizes vary according to chromatin repeats (Ogawa & Okazaki 1980, Smith & Whitehouse 2012). Pol δ synthesizes and displaces much of the Pol α–synthesized RNA/DNA as a 5′ flap that will be subject to further processing (discussed hereafter). Pol δ has higher processivity than Pol α, as it is associated with PCNA, and has higher fidelity and 3′-to-5′ exonuclease activity, allowing for proofreading activity (Prindle & Loeb 2012). Relative to the leading strand, the synthesis of the lagging strand is a far more complicated biochemical process that needs an additional processing step to complete replication.

2.2.3. Lagging-strand processing. After the synthesis of the lagging strand, the Pol α–synthesized 5′ flaps have to be cleaved, the RNA primer has to be removed, and the nicks between the Okazaki fragments must be ligated. RNase H is responsible for removing the RNA primers (Cerritelli & Crouch 2009). There are at least two pathways by which the flap is metabolized. The simplest pathway is the short flap pathway, in which FEN1, an exonuclease, is attached to the Pol δ complex and cleaves the RNA/DNA flap at a single site located at the base of the short flap, leaving just a nick between Okazaki fragments, which is ligated by DNA ligase later on (Bambara et al. 1997). If FEN1 is not associated with the fragment and does not remove the flap before Pol δ displaces a large chunk of DNA, then a long ssDNA flap is formed to which RPA is able to bind. Once RPA binds the long flap, FEN1 is unable to process the flap. To dissociate RPA, a different exonuclease, Dna2, cleaves the long flap, leaving a short flap behind which FEN1 is now able to efficiently process. Interestingly, the helicase Pif1 biases the strands that are normally processed by the short flap pathway to instead be processed via the long flap pathway (Rossi et al. 2008). Replication is complete after the lagging strand is processed, the end result of which is two copies of the original molecule: one produced via leading-strand synthesis and the other produced by lagging-strand synthesis/processing. Although these processes are theoretically identical, the molecular and temporal differences in lagging-strand synthesis/processing have the potential to create molecular differences between the two sister chromatids—differences that may have important functional consequences.

2.3. The Functional Relevance of the Leading- and Lagging-Strand Asymmetries

More recently, studies have focused on not only the mechanisms by which the leading and lagging strands are replicated, but also the effects of the asymmetries generated by these two divergent replication processes. This section discusses the impact that these differences have on patterns of mutagenesis as well as the role that replication asymmetry plays in the process of yeast mating type switching.

2.3.1. The leading and lagging strands shape the mutagenic landscape of DNA. Random mutations created during DNA replication represent a double-edged sword in terms of the survival and stability of both an individual organism and the population as a whole. On the one hand, random mutations represent an indispensable tool for evolutionary change, without which adaptation to environmental changes would be nearly impossible. On the other hand, mutations also represent a great source of chaos in an organism's genome that, if allowed to proliferate in an unchecked manner, could spell doom for both the organism and the population (Furusawa & Doi 1992, 1998). Therefore, as with many aspects of biological systems, a balance is required to achieve the desired degree of both evolutionary flexibility and genomic stability. Interestingly, the

asymmetric structure and replication of the DNA molecule may provide an elegant solution to balancing flexibility and stability by regulating the distribution of mutations within the genome.

Because the discontinuous synthesis of the lagging strand requires the coordinated effort of a greater number of proteins, researchers initially hypothesized that lagging-strand synthesis could generate mutations at a higher rate than leading-strand synthesis. This type of strand-biased mutation is termed disparity mutagenesis, which was initially proposed as a mechanism to allow organisms to achieve a high mutational load without severely compromising an organism's fitness through loss of genome stability (Furusawa 2014). The evolutionary logic of disparity mutagenesis is as follows: A majority of random mutations are concentrated on the lagging strand as a result of discontinuous synthesis. The leading strand, by virtue of continuous synthesis, remains essentially error free. Inside both an organism (diploid) and a population (haploid), this system creates a mixture of stable, leading-strand-synthesized genes and more plastic, lagging-strand-derived genes. The leading-strand genes reliably propagate the parental genetic state, providing stability for the organism and allowing for continued growth and survival under stable environmental conditions with low selective pressure. However, if the environment suddenly changes to much harsher conditions with more competition and higher selective pressure, now the lagging-strand-derived genes provide an opportunity for rapid adaptation to changed environmental conditions.

Researchers demonstrated via modeling that by directing a majority of the mutational load to one side of the replication fork, organisms could survive under a significantly higher mutation rate than if the same degree of mutational load were randomly distributed. Researchers further demonstrated the evolutionary value of disparity mutagenesis by using genetic tools to exacerbate the degree to which the lagging strand acquires novel mutations. Using Pol δ mutants that preferentially increase mutagenesis rates on the lagging strand, researchers found that they could culture yeast in extremely harsh environmental conditions in which wild-type yeast would be unable to adapt and grow (Shimoda et al. 2006, Tanabe et al. 1999). Although early studies of disparity mutagenesis were largely theoretical, they suggested that if certain mechanisms could bias mutation distribution, such a process would be extremely beneficial to an organism's survival and adaptability in a diverse range of conditions (Furusawa 2012). Interestingly, research discussed below has revealed evidence for molecular mechanisms that could generate asymmetries in strand mutational rates.

The divergent synthesis of the leading versus the lagging strand allows for the possibility that these two strands could have different rates or types of mutagenesis. Early studies on this topic relied primarily on the Kunkel mutagenesis method (Kunkel 1985) to introduce individual site-specific mutations in a wide array of genes in both eukaryotic and prokaryotic unicellular organisms as well as in vitro. As the mutations are introduced into sites that can be screened by phenotype or survival, reversion rates of these genes could be measured. From this body of work, it has been concluded that reversions were more common in a wider variety of circumstances from lagging-strand replication relative to leading-strand replication (Francino & Ochman 1997). Some groups have documented that in particular cases the leading strand can be more error prone (Fijalkowska et al. 1998), although transcription could be playing a larger role in these cases than originally thought. The generality is that the lagging strand has higher overall rates of mutagenesis. One reason for the different mutagenesis rates between the two strands could be the different fidelities of DNA polymerases. A recent study found that in both yeast and human cells, approximately 1.5% of the replicated genome was Pol α–synthesized DNA fragments, which are retained near Okazaki junctions at the end of Okazaki fragments. Importantly, this retained Pol α–synthesized DNA underlies the higher mutagenesis rate on the lagging strand due to the lower fidelity of Pol α (Reijns 2015). Pol α–synthesized DNA may be retained at an even higher rate near transcription factor binding sites, creating hot spots of lagging-strand-derived

mutations. Together, these studies indicate that the lagging strand shapes the mutational landscape of cells (Reijns 2015). Furthermore, these findings support the disparity mutagenesis model across multiple domains of life.

One interesting finding originating from studies of mutation distribution is that actively transcribed genes have very different mutation rates depending on whether they are located on the leading or the lagging strand (Liu & Alberts 1995). Leading-strand genes are transcribed in the same direction as the movement of the fork, whereas the lagging-strand genes are transcribed in the direction opposite to that of the fork's progression. Transcribed genes on the lagging strand can be referred to as head-on genes, as the RNA polymerase and DNA replication fork can collide in a head-on orientation. This process can be highly mutagenic, resulting in high rates of insertions and deletions in the gene body and promoter, as well as in high rates of base substitutions specifically enriched at the promoter region (Sankar et al. 2016) (**Figure 2**). Head-on gene orientation was found to result in a 60% increase in the rate of mutagenesis compared to codirectional gene orientation. It is thought that the increased rate of insertions and deletions is due to the replication and transcription machinery collisions, leading to replication fork stalling and fork slippage, which often result in insertions and deletions. The base substitution rate is significantly higher at the promoter region, specifically at the binding site of the sigma factor on the lagging strand. It is currently debated whether increased base substitution at the transcribed region is due to the higher baseline mutation rate of the lagging strand or due to replication-transcription collisions (Sankar et al. 2016). Other groups have demonstrated that the increased rate in lagging-strand mutagenesis with the head-on orientation is transcription dependent, which provides evidence that the higher rate of this mutation in lagging-strand genes is due to replication-transcription collisions (Paul et al. 2013). Hence, the significantly higher mutation rate of the lagging strand is likely due to both incorporation of Pol α–synthesized DNA and head-on replication-transcription collisions.

2.3.2. Asymmetries in mutagenesis could play a key evolutionary role. If we consider that actively transcribed genes on the lagging strand have higher mutagenesis rates compared to actively transcribed genes on the leading strand, it would stand to reason that the asymmetric nature of DNA replication plays a key evolutionary role. For housekeeping genes, where high rates of mutations resulting in amino acid changes would be deleterious, it may be favorable to transcribe these genes on the leading strand. Strikingly, 94% of the essential genes in the genome of *Bacillus subtilis* are, in fact, transcribed on the leading strand (Rocha & Danchin 2003). This suggests that for essential genes, inversions that orient them to be transcribed away from the Ori sequence, and thereby transcribed codirectionally with DNA replication on the leading strand, would be selected for to avoid deleterious mutations resulting from replication-transcription head-on collisions. Although it is debated whether there is a selection for orienting nonessential genes to be transcribed from the lagging strand, these genes do appear to have elevated rates of mutagenesis and undergo accelerated gene evolution, regardless of their orientation on the lagging strand. This could create heterogeneity in populations of unicellular organisms, which could promote survival in changing environmental conditions, as postulated by the disparity mutagenesis hypothesis.

The response to replication-transcription collisions has also been studied in metazoans. In metazoans, origin specification is flexible, and genetic heterogeneity among a population of cells is more deleterious than beneficial. In this context, replication-transcription collisions are avoided far more than in unicellular organisms. Whereas unicellular organisms orient their genomes to passively promote codirectionality, metazoans tend to avoid replication-transcription collisions by spatially and temporally separating transcription and replication (Helmrich et al. 2013). This property is accomplished in a variety of ways for different genes and in different cells. Interestingly, *FHIT*, *WWOX*, and *IMMP2L* genes in human cells are reported to take longer than

one cell cycle to transcribe due to their enormous size. Therefore, they cannot easily separate replication-transcription collisions temporally. As a result, these genes have common fragile sites that are particularly susceptible to DNA double-strand breaks and R-loop formation as a result of replication-transcription collisions. These observations suggest that, in metazoans, replication-transcription collisions can result in major genome instability (Helmrich et al. 2011). Because many S phase transcribed genes have active mechanisms to prevent replication-transcription collisions, flexible origin specification may serve as an additional protection against creating mutagenesis hot spots due to replication-transcription collisions. Dynamic origin specification could minimize the chances that a gene is consistently replicated in a head-on fashion and thus highly mutagenized. Furthermore, many promoters serve as additional sites for preferential ORC binding due to their open chromatin state (Sequeira-Mendes et al. 2009). Localizing origins to promoters of actively transcribed genes is likely to ensure that, even if replication-transcription collisions occur, the origin is more likely to be upstream of the gene, and therefore any replication-transcription collisions are likely to be codirectional, with minimal mutagenicity. Overall, it appears that metazoans use a wide variety of mechanisms to avoid replication-transcription collisions, whereas bacteria minimize fitness-reducing mutations by orienting essential genes to be transcribed codirectionally with replication. Bacteria may additionally use head-on lagging-strand transcription to promote genetic heterogeneity in nonessential genes.

2.3.3. The leading and lagging strands contribute to yeast mating type switching. Asymmetries in DNA replication have the potential to generate a variety of strand-specific differences capable of affecting important cell fate decisions. The mating type switching behavior of yeast has long served as an excellent model system to study the mechanisms of asymmetric cell division: the phenomena by which two daughter cells acquire different fates following division (Haber 2012, Hanson & Wolfe 2017, Holmes et al. 2005). In yeast, mating type refers to a cell's competency to mate with other haploid yeast cells. For instance, in the yeast *Saccharomyces cerevisiae*, only haploid cells with the opposite mating type (a versus α) could mate to form a diploid cell. A similar principle is true for *Schizosaccharomyces pombe*, where mating type plus (P) cells can mate with only mating type minus (M) cells. This mating competency reflects the differential transcription of mating type–related genes in the two different mating yeast groups (a versus α; P versus M) (Haber 2012). Interestingly, mating type is not a fixed identity of yeast. Rather, a yeast cell can alter its mating type over the course of several cell divisions, a process known as mating type switching. Several decades of study in *S. pombe* have revealed that mating type switching is driven by the asymmetries inherent in the process of DNA replication (Dalgaard & Klar 2001). More specifically, the discontinuous lagging-strand synthesis of the mating type locus (*MAT1*) marks one sister chromatid with a molecular lesion, which contributes to mating type switching. The lesion is composed of two RNA nucleotides, likely as the result of imprecise excision of an RNA primer (Klar 1987, 1990, 2010). During the subsequent S phase, this lesion acts as an impediment to the motion of the replication fork as it is moving through the *MAT1* locus (Klar et al. 1991, Nakayama et al. 2001). When the fork collides with the lesion, it causes a stalling event (Yamada-Inagawa et al. 2007). To rescue the stalled fork, the yeast cell undergoes a recombination event similar to synthesis-dependent strand annealing. During this process, DNA from a nearby donor locus (*MAT2* or *MAT3*) is copied into the *MAT1* locus, thereby replacing the original genetic material and resulting in a switch of cellular mating type (Arcangioli & De Lahondés 2000, Egel 2004) (**Figure 3**). Further studies have revealed that the cell actively coordinates replication forks in the *MAT1* region to ensure that this gene is always replicated in a unidirectional manner (Dalgaard & Klar 1999). The primary tool for the cell to control fork progression is a *cis*-acting polar terminator of replication termed *RTS1*, which controls fork progression at the *MAT1* locus

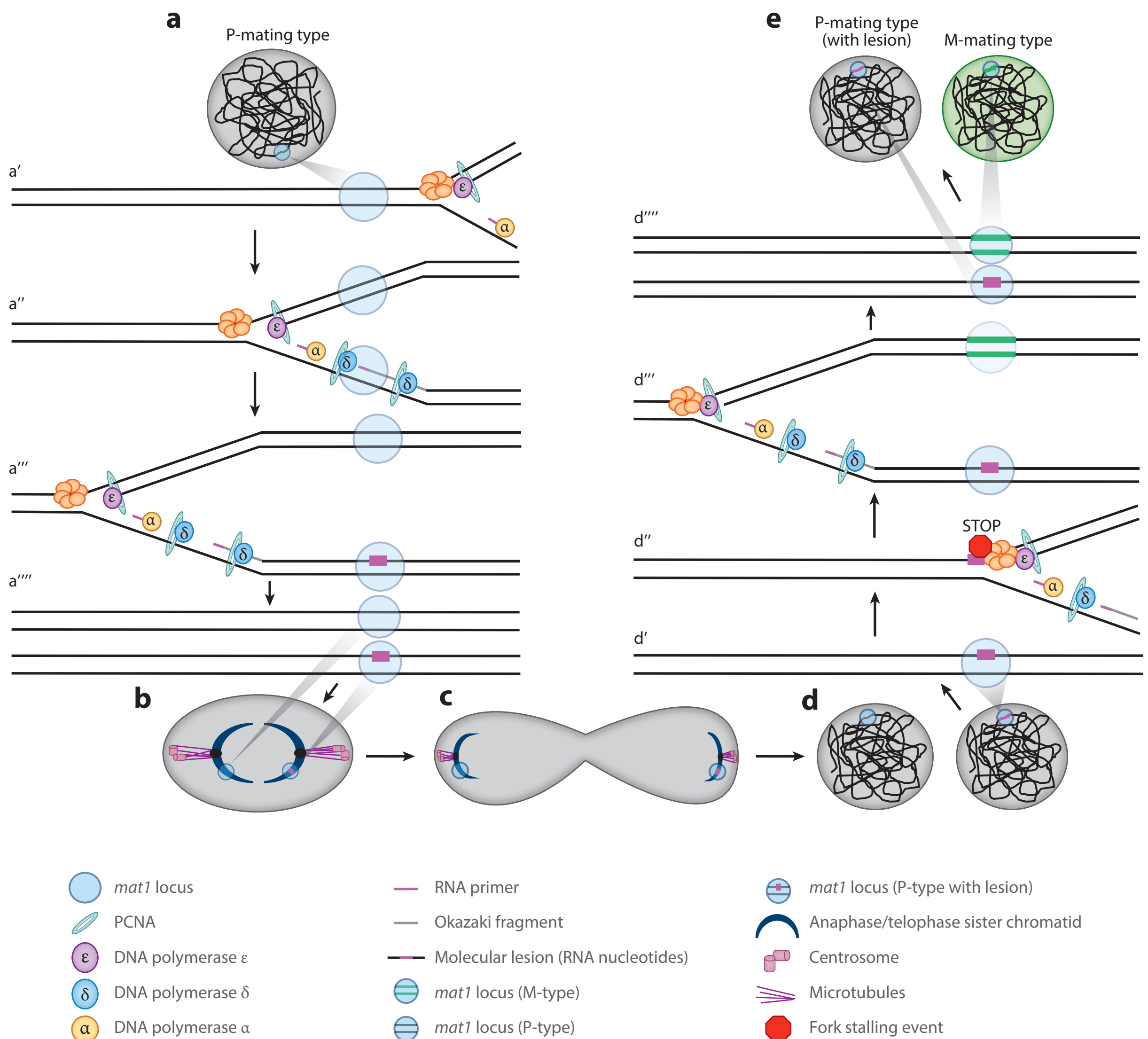


Figure 3

Asymmetries in DNA replication underlie mating type switching in *Schizosaccharomyces pombe*. (*a*) A P-type mating cell entering DNA replication with the *mat1* (mating type) locus labeled with a blue circle. (*a*′) The replication fork approaches the *mat1* locus. (*a*″) As replication proceeds, one strand is replicated by continuous (leading-strand) synthesis, whereas the other strand is replicated by discontinuous (lagging-strand) synthesis, characterized by numerous RNA primers. (*a*‴) One of these primers fails to be properly removed during lagging-strand processing, leaving a molecular lesion (two RNA nucleotides) necessary for mating type switching to occur. (*a*⁗) Two sister chromatids, one with a molecular lesion and one that is unmarked, exist. (*b*,*c*) The two sister chromatids are segregated to distinct daughter cells during mitosis. (*d*) Two daughter cells, one with the molecular lesion and one without, enter S phase. (*d*′) In the daughter cell with the molecular lesion, the replication fork approaches the *mat1* locus. (*d*″) The replication fork stalls when it hits the lesion. (*d*‴) The fork is rescued in a DNA repair mechanism resembling synthesis-dependent strand annealing. The result of this process is that the strand in which the collision occurred has new DNA copied into the *mat1* locus, resulting in a switch in mating type from P to M. (*d*⁗) The production of two sister chromatids: one that has switched mating type and another that now contains a molecular lesion. (*e*) The production of two daughter cells: one that has switched mating type and another that has not switched mating type.

by halting fork progression toward the centromere-distal direction. These findings demonstrate that the asymmetries inherent in DNA replication can be controlled and utilized to ensure robust outcome during asymmetric cell divisions.

3. REPLICATION AND THE CHROMATIN LANDSCAPE

In addition to having impacts on DNA sequences, the asymmetries inherent in the process of DNA replication have important roles in epigenetic regulation (Probst et al. 2009). Epigenetic phenomena refer to modifications made to DNA or DNA-associated proteins that do not alter DNA sequences but lead to heritable changes in gene expression. Many epigenetic phenomena affect changes in gene expression via altering DNA structure, packaging, and accessibility. By doing so, epigenetic phenomena play an essential role in regulating cell fate decisions and are involved in helping to establish and maintain proper cell identity (Allis & Jenuwein 2016). In this section, we discuss prominent epigenetic mechanisms and explore ways in which asymmetric DNA replication may impact the propagation of these molecular marks.

3.1. DNA Methylation

The addition of a single methyl (–CH_3) group to the fifth carbon in the cytosine base (abbreviated 5mC) is one of the best-studied and fully characterized epigenetic signatures known to modulate gene expression. Cytosine methylation has a repressive effect on DNA bearing the epigenetic mark, an effect with a variety of important roles in regulating the chromatin landscape (Kulis & Esteller 2010, Smith & Meissner 2013). For many years, cytosine methylation was believed to be the only DNA modification known to have a specific role in regulating gene expression in eukaryotic genomes (Bird 2002). However, recent studies in *Caenorhabditis elegans* and *Drosophila melanogaster* as well as in human embryonic stem cells have demonstrated the presence of another DNA modification, methylation of the sixth carbon in the adenine base (6mA), which has been shown to have functional consequences in epigenetic regulation (Greer et al. 2015, Wu et al. 2016, Zhang et al. 2015). 6mA has been previously characterized in bacteria, in which it plays important roles in regulating gene expression (Low et al. 2001). Interestingly, deposition of new adenine methylation following replication fork passage appears to have a marked asymmetry in bacterial genomes. Although marks are fully restored on both sister chromatids following DNA replication, the kinetics of mark restoration differ significantly between sisters built via leading-strand synthesis and sisters built via lagging-strand synthesis. Leading strands remethylate nascent DNA strands as many as 2 seconds before the lagging strand, leading to a spatial separation of ~700 nm. It has been hypothesized that the temporal and spatial asymmetries between the leading strand and the lagging strand in terms of the recovery of adenine methylation reflect the additional processes required for complete lagging-strand synthesis (Stancheva et al. 1999). More specifically, researchers conclude that the Okazaki fragments formed during lagging-strand synthesis must be fully ligated before DNA methylation can be fully restored. Although a clear biological role for this phenomenon has yet to be demonstrated, asymmetric DNA methylation may be important during nonrandom sister chromatid segregation, a phenomenon discussed below (Tajbakhsh & Gonzalez 2009, Yamashita 2013).

3.2. Transcription and Chromatin Replication

Transcription factors are an essential class of DNA-binding proteins that recognize and bind to a consensus DNA sequence to help regulate the expression of a specific gene or genes (Spitz & Furlong 2012). During DNA replication, transcription factors must be removed to allow for the

progression of the replication fork. After dissociation, transcription factors must rebind their target sites to maintain proper gene expression. When rebinding, recently dissociated transcription factors are faced with a choice of whether to bind the sister chromatid synthesized via continuous leading-strand synthesis or the sister chromatid synthesized via discontinuous lagging-strand synthesis. Interestingly, recent work has revealed not only that this decision may not be random, but also that this choice may have an important impact on gene regulation and epigenetic inheritance (Alabert & Groth 2012).

Recent studies on chromatin maturation following DNA replication have revealed that the replicative history of leading versus lagging sister chromatids plays an important role in the relative rates of chromatin maturation and resumption of transcription following passage of the replication fork. Interestingly, as described above (**Figure 2**), transcription ahead of the fork also appears to have an important role in regulating fork progression/speed. In cases in which transcription ahead of the fork runs codirectionally with fork progression, chromatin matures faster on the leading strand than it does on the lagging strand, following passage of the replication fork. Interestingly, this faster recovery is dependent on transcription, as the leading and lagging strands mature at comparable rates when transcription is inhibited. Furthermore, when transcription runs counter to fork progression, chromatin matures more quickly on the lagging strand than on the leading strand (Vasseur et al. 2016). These findings are interpreted as follows: When transcription progresses in the same direction as the replication fork, the replication fork will be allowed to move forward, uninhibited by collisions with transcription bubbles. The leading strand, due to the continuous nature of its synthesis, acts as a more capable binding target for the recently dissociated transcription factor. Consequently, the leading strand resumes transcription prior to the lagging strand (**Figure 4**). Conversely, when transcription is oriented counter to the direction of the replication fork, the progression of the replication fork is slowed, thus allowing lagging-strand processing to catch up with the leading strand. Although this model does not clarify why the lagging strand would be favored over the leading strand, it has been proposed that in the immediate aftermath of fork passage, transcription frequently resumes in an asymmetric manner, possibly as a mechanism to buffer gene dosage increases caused by genome duplication during S phase (Vasseur et al. 2016).

Interestingly, recent studies on transcriptional memory in *Drosophila* embryos have revealed that differences in transcription in sister chromatids may be reliably inherited throughout cell divisions. Using a transgene containing a suboptimal *Snail* enhancer element driving a MS2-hairpin reporter, researchers observed that following cell division, daughter cells derived from an actively transcribing mother cell were able to initiate transgene transcription significantly earlier than daughter cells derived from a nontranscribing mother. Furthermore, one of the two daughters of the transcribing mother showed transcription initiation significantly earlier than the other daughter, suggesting that the memory of transcriptional activity is preserved in and inherited by only one of the two sister chromatids. Researchers went on to demonstrate that differences in epigenetic information on sister chromatids are likely the source of the observed asymmetries in resuming transcription following mitosis (Ferraro et al. 2016).

What is the source of epigenetic asymmetries between sister chromatids that regulate asymmetric transcription initiation following mitosis? On the basis of previously discussed findings (Vasseur et al. 2016), DNA replication may play a role in establishing transcriptional asymmetries between sister chromatids. Due to the role that replicative history can have in the resumption of transcription following replication fork passage (described above), asymmetries present during sister chromatid synthesis may play an important role in establishing asymmetric epigenetic states during DNA replication. To explore this intriguing possibility, we consider the following hypothetical example: Following replication fork passage, a codirectional gene reestablishes transcription factor

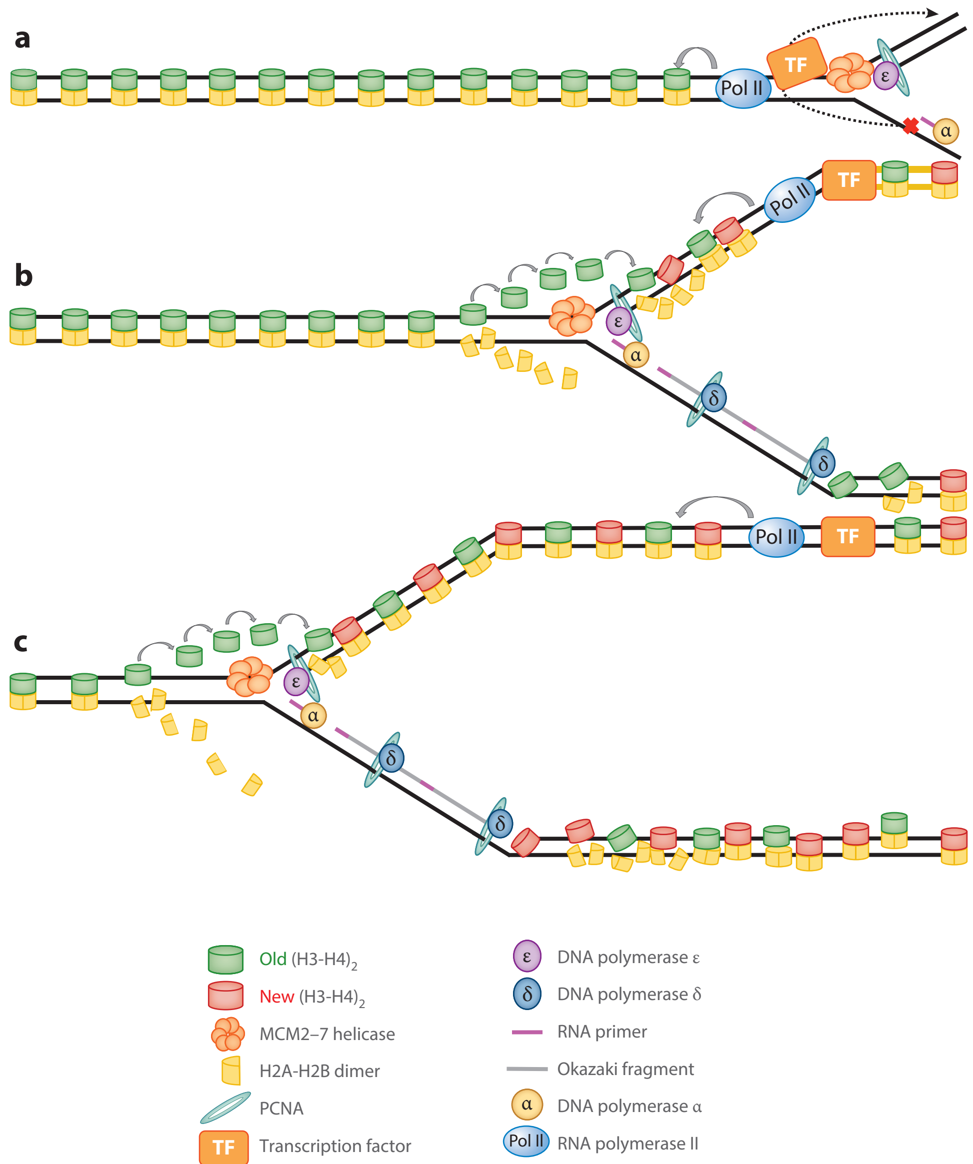
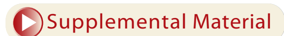


Figure 4

Asymmetries in DNA replication affect chromatin maturation following fork passage. (*a*) The replisome approaches the bound transcription factor, with RNA polymerase II (Pol II) transcribing in the same direction as the fork. The approaching helicase causes the transcription factor to dissociate. The red x indicates high risk of mutation. Due to the fact that the lagging strand is still being processed, the transcription factor will preferentially bind the leading strand. (*b*) Once bound, the transcription factor can initiate transcription, which helps structure and order the nucleosomes on the leading strand. (*c*) The leading strand now has ordered, phased nucleosomes, whereas the lagging strand, due to lack of transcription, still has disordered nucleosomes.

binding and initiates transcription on the leading-strand-synthesized sister chromatid while the complementary lagging-strand-synthesized sister chromatid continues to undergo chromatin maturation (Vasseur et al. 2016). If no transcription factor binds the lagging strand before chromatin fully matures, nucleosomes could invade enhancer/promotor binding sites, thereby reducing the likelihood of transcription initiation (Luebben et al. 2010, Thurman et al. 2012). Furthermore, active transcription on the leading strand could introduce chromatin modifications capable of reinforcing transcriptional activity. Such chromatin modifications could include deposition of histone marks associated with gene activation (H3K4me3), or replacement of H3 with the histone variant H3.3 (Ahmad & Henikoff 2002, Buratowski & Kim 2010, Chen et al. 2013). If the lagging-strand-synthesized sister chromatid fails to reinitiate transcription (or does so at a reduced rate), it is conceivable that these chromatin modifications would never be deposited, leading to their relative enrichment on the leading-strand-synthesized sister (**Supplemental Figure 1**). As both H3K4me3 and H3.3 can be maintained and inherited through mitosis (Muramoto et al. 2010), differences as to both of them between leading- and lagging-strand-synthesized sister chromatids could bias transcriptional activity in daughter nuclei following mitosis. This hypothesis is supported by observations suggesting that, once asymmetries between sister chromatids are established, they can be reliably inherited through mitosis and can impact gene regulation in daughter cells (Ferraro et al. 2016). These findings also hint at the intriguing possibility that cells might use asymmetries during DNA replication to ensure asymmetric transcriptional outcomes in daughter cells.



Although such a mechanism would be ideal for patterning gene expression during asymmetric cell division, a global epigenetic asymmetry between sister chromatids seems ill suited for the stable propagation of the parental epigenetic states during symmetric cell division. More specifically, if the asymmetries inherent in the replication fork can be stably propagated and inherited throughout cell division, how then does a cell ensure that housekeeping genes achieve a more similar level of transcriptional activity post–S phase and postmitotically? One potential answer lies in the role that cohesins may play during S phase to ensure that nucleosome-free regions (NFRs) are not lost (**Supplemental Figure 2**). As transcription factors must compete with histones for binding sites in the eukaryotic genome, a mechanism must be present to ensure that regions normally occupied by transcription factors do not disappear as a result of promiscuous nucleosome incorporation during DNA replication. Recent studies have revealed that, in regions where a host of transcription factors bind, cohesins bind after replication fork passage to maintain NFRs and ensure proper nucleosome organization proximal to transcription factor hot spots (Yan et al. 2013). Given that cohesins encircle both sister chromatids following fork passage, we hypothesize that cohesin binding could serve as a mechanism to buffer any molecular/temporal differences between the two sister chromatids to ensure that both sisters maintain NFRs at key genomic regions. As cohesin binding sites differ among different cell types, different cells may utilize cohesin binding as a way to ensure uniform expression for housekeeping genes and cell type–specific expression for lineage-specific genes. Although it is unclear whether cohesin plays a direct role in buffering strand asymmetries created during DNA replication, studies of cohesin mutants have revealed that loss of cohesin severely disrupts the chromatin landscape and leads to gene misregulation, which has been linked to several human disorders, including Cornelia de Lange syndrome and Coffin-Siris syndrome (Izumi 2016, Nasmyth & Haering 2009, Onn et al. 2008).

3.3. Nucleosomes in DNA Replication

As with other components of chromatin, the process of DNA replication presents a challenge for cells to effectively maintain epigenetic information because nucleosomes must be disassembled and stripped from DNA to allow for progression of the replication fork (McKnight & Miller 1977,

Sogo et al. 1986). The assembly of newly synthesized DNA into chromatin, termed replication-coupled nucleosome assembly, is defined by the coordination of two processes: recycling of old histones and incorporation of new histones (Bannister & Kouzarides 2011, Burgess & Zhang 2013) (**Supplemental Figure 3**). Although significant progress has been made in understanding the process by which new histones are incorporated, much less is known regarding the molecular mechanisms by which old histones are recycled. As old histones contain posttranslational modifications (PTMs) capable of regulating gene expression, a better understanding of histone recycling should provide important insights into understanding how the process of DNA replication impacts epigenetic inheritance. Although the impact that asymmetric DNA replication has on histone recycling has yet to be fully elucidated, the following sections describe current theories and recent experimental data regarding how DNA replication and histone recycling are coordinated.

Supplemental Material

3.4. Three Models for Histone Recycling

Several models have been proposed to explain how old histones are efficiently recycled after passage of the replication fork: the semiconservative model, the dispersive model, and the conservative model (**Figure 5**).

3.4.1. Semiconservative model of histone recycling. The histone octamer is composed of one $(H3\text{-}H4)_2$ tetramer and two (H2A-H2B) dimers. As a majority of the PTMs known to regulate gene expression have been found on the N-terminal tails of H3 and H4, many studies of histone inheritance have focused primarily on understanding the behavior of the $(H3\text{-}H4)_2$ tetramer as it is displaced and recycled during passage of the replication fork (Hammond et al. 2017). The quasi-symmetry of the histone octamer initially prompted researchers to propose the semiconservative model of histone inheritance, in which the $(H3\text{-}H4)_2$ tetramer would be split into two dimers, each of which would be inherited by newly synthesized sister chromatids. The model further postulated that each of the (H3-H4) dimers would contain the relevant epigenetic information necessary to propagate the parental chromatin state to each of the newly synthesized sister chromatids (Zhu & Reinberg 2011) (**Figure 5*a***). However, recent findings suggest that semiconservative histone inheritance is not the primary means by which epigenetic information is passed from the mother strand to each of the newly synthesized daughter strands. Mass spectrometry analysis of preexisting and newly synthesized histones has revealed several key features of the $(H3\text{-}H4)_2$ tetramer that argue against a semiconservative mode of histone inheritance: First, $(H3\text{-}H4)_2$ tetramers infrequently split, meaning that mixed tetramers comprising old and new (H3-H4) dimers rarely ever form. Second, the (H3-H4) dimers that compose the $(H3\text{-}H4)_2$ tetramers are not symmetrically modified (Chen et al. 2011, Van Rossum et al. 2012, Voigt et al. 2012). Thus, even in the case of a rare $(H3\text{-}H4)_2$ splitting event, the likelihood of both (H3-H4) dimers containing the same PTMs is extremely low (Xu et al. 2010). Interestingly, tetramers containing the histone variant H3.3 show a higher frequency of splitting than do H3-containing tetramers (~23% for H3.3 versus ~3% for H3). Furthermore, these splitting events appear to be associated with cell type–specific enhancer elements (Huang et al. 2013), suggesting that, although semiconservative histone inheritance is unlikely to regulate epigenetic inheritance on a genome-wide scale, semiconservative histone inheritance could play an important role in propagating epigenetic information at particular genomic loci.

3.4.2. Dispersive model of histone recycling. If semiconservative histone inheritance is confined to very limited regions of genome replication, how then are the vast majority of histones recycled during DNA replication? A majority of results support the dispersive model (Alabert &

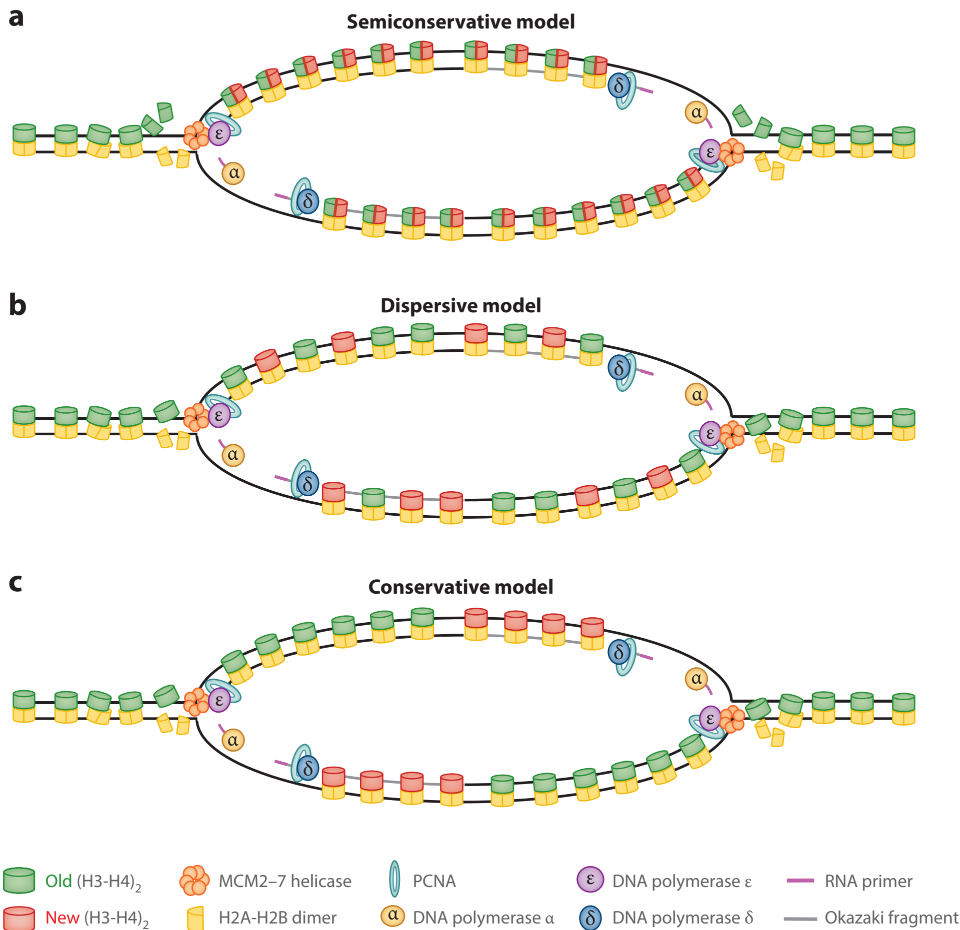


Figure 5

Different models for histone inheritance. (*a*) Semiconservative model: Old $(H3\text{-}H4)_2$ tetramers are split at the replication fork, allowing (H3-H4) dimers to be inherited equally on both the leading strand and the lagging strand. New (H3-H4) dimers pair with old (H3-H4) dimers to recreate the tetramer structure. (*b*) Dispersive model: Old $(H3\text{-}H4)_2$ tetramers remain together at the fork and are randomly segregated to the leading strand or the lagging strand in roughly equal numbers. New histones fill in gaps left by old histones to reconstitute nucleosome density. (*c*) Conservative model: Old $(H3\text{-}H4)_2$ tetramers remain unsplit at the fork and are inherited as a tetramer. In this model, tetramers are biased in their inheritance such that either the leading strand (shown) or the lagging strand (not shown) inherits a majority of the old $(H3\text{-}H4)_2$ tetramers. New histones fill in gaps left by old histones to reconstitute nucleosome density.

Groth 2012; Alabert et al. 2015; Jackson & Chalkley 1981, 1985; Hammond et al. 2017; Herz et al. 2014) (**Figure 5*b***). According to this model, $(H3\text{-}H4)_2$ tetramers dislodged by the advancing helicase remain intact during the process of nucleosome breakdown and histone recycling. Old $(H3\text{-}H4)_2$ tetramers are segregated randomly at the fork such that approximately half of the $(H3\text{-}H4)_2$ displaced from the parental strand are recycled to the leading strand and the other half are incorporated onto the lagging strand (**Figure 5*b***). New histone incorporation is necessary to maintain nucleosome density. As new histones are largely devoid of PTMs (Lin et al. 2016), old histones must play an instructive role by assisting new histones to acquire the appropriate

epigenetic signatures needed to recapitulate the parental chromatin state. In support of this hypothesis, chromatin maturation studies have demonstrated that, for a variety of PTMs, old histones are able to recruit the appropriate histone-modifying enzymes (epigenetic writers) to establish the correct PTMs on newly synthesized histones (Alabert & Groth 2012, Alabert et al. 2015, Audergon et al. 2015, Ayyanathan et al. 2003, Hansen et al. 2008, Margueron et al. 2009, Ragunathan et al. 2015). In summary, the dispersive model argues that, by randomly segregating old histones to both newly synthesized sister chromatids, the epigenetic identity of each genomic region can be maintained and propagated to each daughter cell during cell division.

3.4.3. Conservative model of histone recycling. Intriguingly, evidence from several studies suggests that histone inheritance may not always proceed in a dispersive fashion. Several studies into the question of chromatin inheritance have demonstrated that old histones are incorporated in a nonrandom manner, displaying a strand preference during recycling events (Leffak et al. 1977, Riley & Weintraub 1979, Seale 1976, Weintraub 1976). Data from these studies support the conservative model of histone segregation, which hypothesizes that $(H3\text{-}H4)_2$ tetramers are biased in their incorporation at the fork such that either the leading strand (Seidman et al. 1979) (**Figure 5*c***) or the lagging strand (Roufa & Marchionni 1982) preferentially inherits more of old $(H3\text{-}H4)_2$ tetramers recycled from the parental strand. Surprisingly, research efforts have demonstrated directed segregation of old histones to either the leading strand (Seidman et al. 1979) or the lagging strand (Roufa & Marchionni 1982). Although it is unclear why the results of these studies differ, taken together these findings suggest that the process of histone recycling can coordinate with the asymmetries inherent in DNA replication to produce a variety of distinct histone inheritance patterns. Indeed, the fact that evidence can be found supporting both conservative and dispersive models of histone segregation raises an important question concerning the process of histone inheritance; specifically, is histone recycling conserved across all chromatin landscapes, or can the process be regulated and altered on the basis of variable conditions such as cell type and chromatin context? Much in the same way that semiconservative histone segregation can be detected at specific genomic regions enriched with histone H3.3 (Huang et al. 2013), at certain genomic loci, conservative histone segregation may be utilized to generate localized asymmetries in epigenetic information. We speculate that for cells undergoing asymmetric cell division, localized asymmetries at key developmental loci could serve as a valuable intrinsic mechanism to ensure that divergent cell fates arise. In the following section, we discuss several lines of evidence suggesting that DNA replication may be playing a causal role in shaping the epigenetic landscape in preparation for asymmetric cell division.

3.5. Replication Asymmetries Pattern Cell Fate Decisions in *Caenorhabditis elegans*

Research efforts in *C. elegans* development have revealed an asymmetric cell division that may utilize molecular asymmetries associated with replication-coupled nucleosome assembly to generate distinct cell fates. A mutagenesis screen for factors affecting cell fate decisions during *C. elegans* development has identified a gain-of-function mutation in an H3-encoding gene (*his-9*) that is sufficient to disrupt neuronal bilateral asymmetry, which is required for determining the MI motor neuron and the e3D pharyngeal epithelial cell (Nakano et al. 2011, Sulston et al. 1983). The *his-9* (Q125ochre) mutation described by Nakano et al. (2011) eliminates two residues, leucine 126 and isoleucine 130, which are believed to play an important role in the H3-H3 interaction critical for $(H3\text{-}H4)_2$ tetramer formation. Perturbation of the $(H3\text{-}H4)_2$ tetramer structure via the Q125ochre mutation results in H3 gain-of-function activity that disrupts CAF-1-mediated nucleosome

Supplemental Material

assembly at the replication fork (**Supplemental Figure 3**). A similar loss of neuronal asymmetry phenotype could be recapitulated upon knocking down either components of the worm CAF-1 complex or PCNA, a finding that further implicates the process of replication-coupled nucleosome assembly in specifying divergent cell fates. These results suggest that replication-coupled nucleosome assembly has an essential role in regulating asymmetric cell fate decision during *C. elegans* development (Nakano et al. 2011).

Although the precise mechanisms behind the loss of asymmetric cell fate have yet to be elucidated, Nakano et al. (2011) hypothesize that asymmetries during DNA replication, specifically nucleosomal density differences existing between the leading and lagging strands, may underlie the divergent cell fate choices between MI and e3D cells. The elevated density of PCNA molecules present on the lagging strand has led some to propose that the lagging strand may contain a higher nucleosomal density relative to the leading strand (Shibahara & Stillman 1999, Waga & Stillman 1998, Yu et al. 2014). The authors speculate that if nucleosomal density differs at critical developmental genes, such a density difference could drive differential gene expression and distinct cell fate decisions during development (**Supplemental Figure 4**).

3.6. Asymmetric Histone Inheritance in the Germline of *Drosophila melanogaster*

In addition to changes in nucleosomal density, many other aspects of chromatin structure germane to cell fate specification could be affected via replication-coupled nucleosome assembly. Recent work has demonstrated that sister chromatids enriched with distinct histone populations are asymmetrically segregated during asymmetric cell division of *D. melanogaster* male germline stem cells (GSCs). Using a dual-color strategy to label preexisting versus newly synthesized histones, researchers observed that the preexisting H3 is segregated to the male GSCs, whereas newly synthesized H3 is enriched toward the differentiating daughter cell. Subsequent studies have further demonstrated that disruption of asymmetric histone inheritance can lead to adverse phenotypes ranging from cell death to tumorigenesis. Interestingly, asymmetric histone inheritance is specific to the canonical H3, but not to histone variant H3.3 (**Figure 6**). Because H3 is

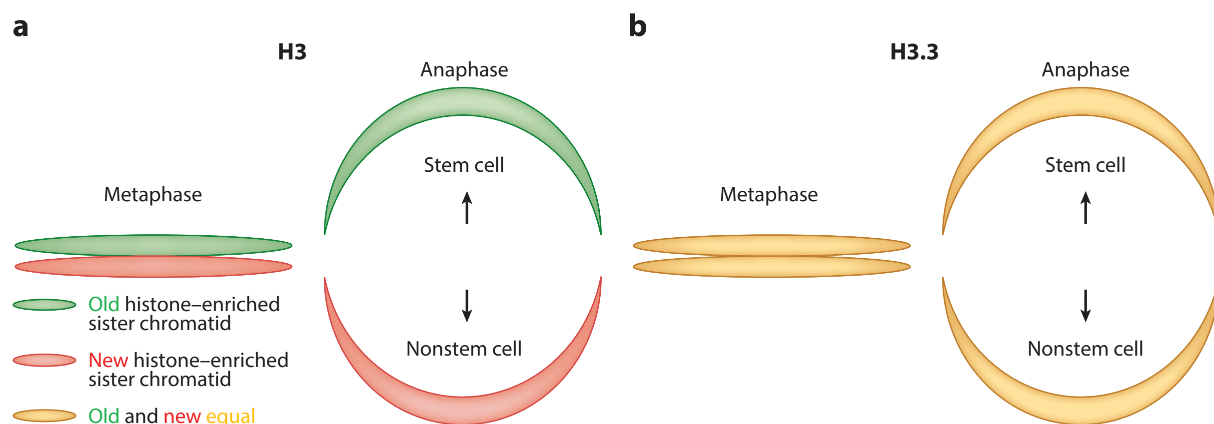


Figure 6

Asymmetric versus symmetric histone distribution in *Drosophila melanogaster* germline stem cell (GSC) sister chromatids. (*a*) Old H3 are enriched in the sister chromatids inherited by the cell fated to remain a GSC, whereas newly synthesized H3 are enriched in the sister chromatids inherited by the daughter cell destined to differentiate. (*b*) Old and new H3.3 are present in equal quantities on all sister chromatids and are inherited equally by GSC and non-GSC daughter cells.

incorporated during DNA replication whereas H3.3 is incorporated in a replication-independent manner, these findings suggest a role for DNA replication in establishing epigenetic information differentially in preparation for asymmetric cell division (Tran et al. 2012).

These findings suggest that, similar to the mechanisms concerning neuronal cell fate specification described above, asymmetries present in the synthesis of the leading strand versus the lagging strand could be an initial mechanism to bias histone inheritance during DNA replication. As has been hypothesized and previously described for transcription factor binding, the temporal and molecular asymmetries that define continuous (leading-strand) versus discontinuous (lagging-strand) synthesis could serve as a mechanism to influence the binding of histone proteins following DNA replication. As discussed above, synthesis and processing of the lagging strand require the combined action of many more proteins than for the leading strand. Consequently, the lagging strand (as its name implies) takes longer than the leading strand to generate dsDNA that is capable of being folded around the histone octamer to form the nucleosome structure. The leading strand, in contrast, emerges from the replicative polymerase fully capable of serving as a landing site for histone proteins recently dislodged by the advancing helicase. Whether by the aid of histone chaperones or by hopping from unreplicated strand to replicated strand, if lagging-strand processing were sufficiently delayed, old histones would be biased in their reassociation with DNA to preferentially bind the leading strand (Annunziato 2013, Brennan et al. 2016, Huang et al. 2015). An important consequence of this binding is that newly synthesized histones would, by default, preferentially incorporate onto the lagging strand. As histone proteins represent a key component of the epigenome, such a mechanism would mean that intrinsic asymmetries present in DNA replication may play an important role in coordinating cellular processes and regulating cell fate decisions during metazoan development.

3.7. Selective Sister Chromatid Segregation

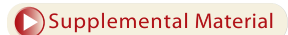
The asymmetric segregation of sister chromatids enriched with distinct histone species represents a unique example of a previously described phenomenon known as selective sister chromatid segregation, which refers to nonrandom segregation of sister chromatids during mitosis. This phenomenon has been observed in a variety of cell types from different organisms. To begin to understand the biological relevance of selective sister chromatid segregation, several models have been proposed: the immortal strand hypothesis (Cairns 1975), the silent sister chromatid hypothesis (Lansdorp 2007), and the strand-specific imprinting and selective chromatid segregation model (Klar 1994, 2008, 2014). All of these models are similar in that they involve nonrandom segregation of sister chromatids during mitosis. The primary difference among these models lies in the molecular features responsible for distinguishing sister chromatids during the process of selective segregation.

The immortal strand hypothesis states that the age of the template serves as the primary feature responsible for differentiating sister chromatids during the process of selective segregation. The theory goes on to posit that by segregating by age of the template strand, continuously dividing populations of cells (stem cells) are able to minimize the accumulation of deleterious mutations that could compromise cellular function (Cairns 1975, 2006). In the context of the immortal strand hypothesis, selective sister chromatid segregation serves a protective role: safeguarding the integrity of the stem cell genome by segregating mutation-prone newly synthesized DNA to differentiating daughter cells. Data supporting the immortal strand hypothesis have come from organisms ranging from plants and fungi to mouse and human tissues (stem cell systems) (Conboy et al. 2007, Lark et al. 1966, Rando 2007, Yennek & Tajbakhsh 2013). However, evidence also exists that not all stem cell systems undergo the nonrandom template strand segregation associated

with the immortal strand hypothesis, suggesting that immortal strand segregation is not a universal feature of stem cells (Comings 1970, Geard 1973, Kuroki & Murakami 1989).

Two additional models, the silent sister chromatid hypothesis and the strand-specific imprinting and selective chromatid segregation model, argue that, rather than having a protective role, selective sister chromatid segregation has a more instructive role during the processes of development and tissue homeostasis. In both models, sister chromatids carrying distinct epigenetic signatures at specific genomic loci are segregated asymmetrically as a mechanism to bias cell fate outcomes, specifically in the case of asymmetric cell division events (Lansdorp 2007, 2012; Klar 1994, 2008, 2014). Recent studies in both the *Drosophila* male GSC system and the mouse satellite cell system have shown biased sister chromatid segregation during stem cell asymmetric cell divisions (Rocheteau et al. 2012, Yadlapalli & Yamashita 2013). However, this bias applies to either particular chromosomes or all chromosomes, suggesting a potential chromosome-specific regulation(s).

Further studies in mouse ES cells suggest that epigenetic differences are the primary means by which sister chromatids are selectively segregated (Klar 1994, 2008, 2014) (**Supplemental Figures 5** and **6**). Through genetic manipulation specifically of mouse chromosome 7, researchers demonstrated that biased sister chromatid segregation patterns appear in a cell type–specific manner. The authors of this study further suggest that these sister chromatids contain differential epigenetic information and that these unique segregation patterns may serve as a mechanism of pattern formation during development (Armakolas & Klar 2006). Furthermore, these researchers demonstrated that these unique segregation patterns are dependent on the *left-right dynein* gene, leading the authors to implicate the mitotic machinery in playing a key role in biased sister chromatid segregation (Armakolas & Klar 2007). However, in these disparate systems it is unclear how distinct sister chromatids are differentially marked and recognized in a manner that would ensure their proper segregation during mitosis. One possible mechanism involves asymmetric DNA replication at the centromeric regions of asymmetrically segregating chromosomes.



In metazoans, the centromere is an epigenetically defined structure marked by the deposition of the histone H3 variant CENP-A, which regulates sister chromatid segregation during mitosis (McKinley & Cheeseman 2016). Asymmetries inherent in the process of DNA replication have been invoked as a possible mechanism by which sister chromatid centromeres could be asymmetrically marked to allow for selective segregation during mitosis. The temporal and molecular differences present in the continuous synthesis of the leading strand versus the discontinuous synthesis of the lagging strand could provide asymmetrically dividing cells with the necessary tools to mark sister chromatids in a molecularly distinct fashion, likely through the asymmetric segregation of key chromatin factors. It has further been proposed that, to ensure uniform asymmetry across the centromeric region, replication forks could be biased in their progression to ensure that one of the two sisters is replicated in a predominantly leading-strand mode of synthesis, whereas the other sister chromatid is replicated largely via discontinuous lagging-strand synthesis (Lew et al. 2008). Whether centromeric regions are replicated in such a coordinated fashion has yet to be demonstrated, but investigations have revealed that epigenetic asymmetries at the centromeric region do underlie the proper recognition and segregation of sister chromatids during asymmetric cell division of the *Drosophila* male GSC (**Supplemental Figure 6**). Researchers recently demonstrated that a pericentromere-enriched, mitosis-specific phosphorylation of threonine three in H3 (H3T3P) selectively labels old histones during the transition from prophase to metaphase (Lin et al. 2016, Xie et al. 2015). Furthermore, the authors found that this asymmetry serves as a mechanism to allow the mitotic spindle to recognize sister chromatids enriched with different populations of histones so as to assure their proper segregation during GSC asymmetric cell division. In spite of H3T3P's prominent role in regulating histone inheritance during asymmetric

cell division in the *Drosophila* germline, the source and molecular mechanisms underlying H3T3P asymmetry remain unclear.

4. CONCLUSIONS: ASYMMETRY OF DNA REPLICATION AS A TOOLKIT

Life accomplishes a huge diversity of tasks by using simple building blocks, organized and manipulated through millennia of selective pressures to ever more precisely match form and function. The structure of DNA represents one of these building blocks. In the past, we gained a great appreciation for how the structure of the dsDNA molecule allows it to serve the essential function of genome duplication. Recent work has given us an appreciation for how the structure of DNA may be functioning to assist in other processes necessary for growth and development. By virtue of the structure of DNA, the process of DNA replication is able to serve as a fundamental tool for allowing life to create heterogeneity. In unicellular organisms, the asymmetry of DNA replication is used to drive gene evolution, maximizing potentially beneficial mutations and limiting deleterious mutations, and can even be used to drive processes such as mating type switching in yeast. In metazoans, DNA replication contributes to the crucial generation of asymmetric cell fates in asymmetric stem cell division and development. Going forward, it will be essential to test these hypotheses more rigorously to determine the extent to which asymmetries present during DNA replication impact asymmetric cell fate outcomes essential to growth and development.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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