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Eukaryotic Genome Instability in Light of Asymmetric DNA Replication

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Abstract

The eukaryotic nuclear genome is replicated asymmetrically, with the leading strand replicated continuously and the lagging strand replicated as discontinuous Okazaki fragments that are subsequently joined. Both strands are replicated with high fidelity, but the processes used to achieve high fidelity are likely to differ. Here we review recent studies of similarities and differences in the fidelity with which the three major eukaryotic replicases, DNA polymerases α , δ and ϵ , replicate the leading strand and lagging strands with high nucleotide selectivity and efficient proofreading. We then relate the asymmetric fidelity at the replication fork to the efficiency of DNA mismatch repair, ribonucleotide excision repair and topoisomerase 1 activity.

key terms

DNA mismatch repair; exonucleolytic proofreading; polymerase; mutation; replication fork; genomic ribonucleotide

Introduction

Genetic information is encoded in long chains of deoxyribonucleic acid (DNA). DNA is made when a DNA polymerase links the 5'-phosphate of a deoxyribonucleoside triphosphate (dNTP) to the 3'-hydroxyl of the deoxyribose at the end of a growing chain (Kornberg and Baker, 1992). As a consequence, each DNA strand has directionality, with one 5'-terminus and one 3'-terminus. The two DNA strands contain complementary bases that assemble into an antiparallel DNA double helix (Watson and Crick, 1953) that provide a means of accurate replication through DNA polymerase-mediated complementary pairing of adenine (A) with thymine (T) and of guanine (G) with cytosine (C). Cellular organisms initiate DNA synthesis at origins of replication within the double-stranded DNA genome, and they replicate the two strands in a coordinated fashion with two replication forks proceeding from each origin, one in each direction. Eukaryotes, Archaea, and certain Bacteria use multiple origins and thus can bring tens, hundreds, or even thousands of polymerases to bear during replication of their genomes. In each fork, one strand, the leading strand, is largely replicated

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continuously. The other, lagging strand is replicated by repeated priming and DNA synthesis of discontinuous fragments (Kainuma-Kuroda and Okazaki, 1975). These Okazaki fragments are ultimately processed into a continuous lagging strand.

This review considers the mechanistic basis for the fidelity of asymmetric replication of the undamaged, eukaryotic nuclear DNA genome, with an emphasis on recent studies. We first consider how the nuclear genome is replicated asymmetrically by three major DNA polymerases (replicases). We then consider emerging information on misincorporation during replication by these replicases that can generate mutations, in the form of base substitutions, insertions and deletions, and can also result in incorporation of ribonucleotides into DNA. We then focus on how misincorporation rates during asymmetric replication relate to differences in three post-replication processes that contribute to genome stability, i.e., DNA mismatch repair (MMR), ribonucleotide excision repair (RER), and topoisomerase I activity.

Eukaryotic nuclear DNA replication is asymmetric

Unperturbed nuclear genome replication primarily involves the action of three major replicases, DNA polymerases (Pols) α , δ , and ϵ (reviewed (Pursell and Kunkel, 2008); Fig. 1; Table 1). These replicases are multi-subunit, B family enzymes that share the ability to catalyze consecutive dNTP incorporation reactions without dissociating from DNA, and to complete replication with high accuracy in the time allotted for cell division. Accuracy is achieved first by selecting correct deoxyribonucleotides for insertion over ribonucleotides or mismatched deoxyribonucleotides (nucleotide selectivity; Fig. 2A). Mismatched nucleotides that are incorporated into the nascent strand may be removed during replication by the proofreading exonuclease activity sites of Pols δ and ϵ (Fig. 2B–F). Mismatches that escape both selectivity and proofreading can be detected and repaired by post-replication MMR (Fig. 2G–K). Lapses in any of these three steps cause genomic instability, which can facilitate evolution but can also initiate and promote disease (Abbas et al., 2013, Heitzer and Tomlinson, 2014, Kunkel, 2011, Arana and Kunkel, 2010).

Studies to date indicate that the four-subunit Pol α holoenzyme (Table 1) contains RNA primase activity that synthesizes an approximately 10 nucleotide RNA primer to initiate replication at origins (orange in Fig. 1). This is followed by synthesis of about 10 to 20 nucleotides of DNA by Pol α 's DNA polymerase (red in Fig. 1). The remaining DNA synthesis for each ~200-base long Okazaki fragment during lagging strand replication is carried out by Pol δ (green in Fig. 1 and see below). Pol α lacks intrinsic proofreading activity and therefore cannot proofread any mismatches it generates. Pol α synthesis is critical for initiation of leading and lagging strand synthesis at origins. But given that chromatin organization quantizes eukaryotic Okazaki fragment lengths into one- to three-nucleosome units (approximately 170–510 bp, and skewed toward the smaller size range (Smith and Whitehouse, 2012)), most Pol α synthesis takes place during synthesis of the lagging strand.

Pols δ and ϵ are responsible for the bulk of replicative synthesis of the undamaged nuclear genome. When these enzymes were discovered, they were suggested to operate on opposite

DNA strands in eukaryotic cells (Morrison et al., 1990). Shortly thereafter, Pol δ , but not Pol ϵ , was demonstrated to be required for replicating the mammalian SV40 virus *in vitro* (Waga and Stillman, 1994). Thereafter, *Saccharomyces cerevisiae* lacking Pol ϵ catalytic and exonuclease activities (*pol2-16*) was demonstrated to be viable (Dua et al., 1999, Kesti et al., 1999, Feng and D'Urso, 2001, Ohya et al., 2002). These studies clearly show that in the absence of catalysis by Pol ϵ , Pol δ and/or Pol α can synthesize both the leading and the lagging DNA strands. This replication model remains of active interest, as exemplified by a recent study implying that Pol δ is the major replicase for both the leading and lagging strands ((Johnson et al., 2015); reviewed (Stillman, 2015)). In this model, the polymerase activity of Pol ϵ is not important for the bulk of DNA replication, but its 3'-exonuclease activity is important for editing errors made by Pol δ during leading strand replication. Readers interested in this model are encouraged to read these articles.

However, observations from multiple groups suggest an alternative possibility, namely that during replication of undamaged DNA in eukaryotes, the polymerase activities of Pols δ and ϵ are primarily responsible for replicating different DNA strands. Evidence obtained nearly 20 years ago suggested that *S. cerevisiae* Pols δ and ϵ proofread base analog-induced DNA replication errors on opposite DNA strands (Shcherbakova and Pavlov, 1996), and mutation spectra in Pol δ and ϵ exonuclease-deficient mutants differed sufficiently by reporter gene orientation to suggest that Pol δ wasn't solely responsible for replicating both strands (Morrison and Sugino, 1994, Karthikeyan et al., 2000). In 2006, genetic evidence suggested that *S. cerevisiae* Pol δ was able to proofread Pol α errors and Pol ϵ was not (Pavlov et al., 2006). In the next two years, yeast replicase derivatives were described whose properties were consistent with a model in which Pols δ and ϵ are primarily responsible for lagging and leading strand synthesis, respectively (Figure 1). Pols α , δ and ϵ all discriminate against ribonucleotide incorporation into DNA by way of a strictly-conserved "steric gate" amino acid that clashes with the 2'-hydroxyl of an incoming rNTP (Joyce, 1997, Brown and Suo, 2011, Williams and Kunkel, 2014). Pol α , δ , and ϵ variants with mutations at or immediately adjacent to the steric gate tyrosine in the polymerase active site often have mutator phenotypes and reduced ribonucleotide discrimination. For the sake of simplicity, such variants will hereafter be referred to as mutator variants. Using an orientation-dependent mutation reporter assay, one study showed that near origin *ARS306* on *S. cerevisiae* chromosome III, a Pol ϵ mutator variant produced mutation hotspots consistent with synthesis of the nascent leading strand (Pursell et al., 2007). A year later, the study of a Pol δ variant reported mutation biases implying that Pol δ is the primary lagging strand replicase (Nick McElhinny et al., 2008). Since then, mutation patterns in *Schizosaccharomyces pombe* strains with variant replicases (Miyabe et al., 2011), and genome-wide studies of replication errors in *S. cerevisiae* strains (Larrea et al., 2010, Lujan et al., 2012, Lujan et al., 2014) also map Pol δ to the lagging strand and Pol ϵ to the leading strand. In addition, Pol ϵ exonuclease-defective human tumors have mutational patterns near origins that are similar to those in cell extracts, but only if Pol ϵ is assumed to work on the leading strand (Shinbrot et al., 2014). Support for this model also comes from eSPAN (enrichment and sequencing of protein-associated nascent DNA), which uses immunoprecipitation of BrdU-labeled nascent DNA after chromatin immunoprecipitation (ChIP) to discover which nascent strand of a given replication fork physically associates with a given replication protein (Yu et al., 2014).

Results obtained using this technique are in agreement with the mutation analyses mentioned above, and suggest a model wherein Pol ϵ primarily replicates the leading strand and Pol δ primarily replicates the lagging strand.

Recent work indicates that the division of labor among the three replicases is established by divergent interactions with accessory proteins. Pols δ and ϵ appeared to load on primer termini via separate mechanisms (Johansson and Macneill, 2010, Chilkova et al., 2007). The eSPAN results mentioned above indicate that Pol α , Rfa1 (one component of the RPA single-stranded DNA-binding complex), and Rfc1 (a component of RFC, the PCNA loading complex) are enriched on the lagging strand. At the same time, Cdc45, Mcm6 (components of the CMG replicative helicase) and Mcm10 localize to the leading strand, reinforcing predictions that the helicase travels with the leading strand in active replication forks (Fu et al., 2011). The physical interaction between Pol ϵ and CMG, and Pol ϵ inefficiency in the absence of CMG or a CMG-interacting subunit of Pol ϵ , have lead to a proposal that Pol ϵ and CMG are a 15-subunit leading-strand holoenzyme (Langston et al., 2014). CMG also excludes Pol δ from the leading strand (Georgescu et al., 2014, Georgescu et al., 2015), suggesting that CMG establishes and maintains replicase/strand asymmetry.

Generating and proofreading mismatches during leading and lagging strand replication

Because replication of the two DNA strands is asymmetric, replication fidelity in the absence of mismatch repair is predicted to also be asymmetric. Numerous studies are consistent with this prediction. Pol α lacks proofreading activity and is the least accurate of the three eukaryotic replicases during DNA synthesis *in vitro* (Kunkel, 2009). In the absence of MMR, variants of yeast Pol α with amino acid substitutions in the polymerase active site readily generate point mutations *in vitro* and *in vivo* (Niimi et al., 2004, Nick McElhinny et al., 2008, Lujan et al., 2013). Moreover, the Pol α variant preferentially generates errors at replication origins and during synthesis of the lagging strand (Lujan et al., 2013, Nick McElhinny et al., 2008, Waisertreiger et al., 2012). In comparison, Pols δ and ϵ can both proofread replication errors, and they are more accurate than Pol α *in vitro* (e.g., see (Fortune et al., 2005, Shcherbakova et al., 2003), reviewed (Kunkel, 2009)). As mentioned above, mutator alleles of yeast Pol δ and Pol ϵ containing amino acid substitutions in their polymerase active sites also generate replication errors *in vivo*. In MMR-deficient (*msh2*) cells, these variants generate base substitution and indel mutations with patterns that are consistent with the primary roles of Pols δ and ϵ in synthesis of undamaged DNA during lagging and leading strand replication, respectively (Larrea et al., 2010, Lujan et al., 2013, Lujan et al., 2012, Miyabe et al., 2011, Nick McElhinny et al., 2008, Pursell et al., 2007).

In the absence of proofreading and MMR of base substitutions (*msh6*), studies of replication error specificity *in vivo* (St Charles et al., 2015) suggest that exonuclease-deficient yeast Pols δ and ϵ have apparent nucleotide selectivities of $>10^6$ and $>10^7$, respectively. That is, an incorrect dNTP is selected less than once per million bases synthesized by Pol δ and per ten million bases synthesized by Pol ϵ . Thus both polymerases are highly accurate. A comparison of these rates to the rates in cells that are deficient in only MMR (St Charles et al., 2015) indicates that base-base mismatches are also efficiently proofread by Pols δ and ϵ . Many of these mismatches are likely to be proofread intrinsically

by the exonuclease activity of the polymerase that made the mismatch (Figure 2, panels C and E). Some mismatches may be extrinsically proofread by the exonuclease activity of another replicase (Figure 2, panels C, D and F). Diploid strains with defects in both exonucleases (*pol3-01* and *pol2-4* mutations) have synergistic increases in mutation rate, implying that the two exonucleases act in series (i.e. compete for some of the same mismatches) and/or that loss of both exonucleases saturates MMR (Morrison et al., 1993, Morrison and Sugino, 1994). Genetic evidence suggests that Pol δ , but not Pol ϵ , proofreads errors made by Pol α , which does not possess intrinsic proofreading (Pavlov et al., 2006). A more recent study indicates that Pol δ can proofread errors made by Pol ϵ , but that Pol ϵ , which can intrinsically proofread its own errors, does not extrinsically proofread errors made by another polymerase (Flood et al., 2015). The latter point contradicts the proposal by Johnson et al. that Pol ϵ proofreads errors made by Pol δ (Johnson et al., 2015), indicating that more work will be required to sort this out.

S. cerevisiae Pol ϵ has a different error signature *in vitro* than does *S. cerevisiae* Pol δ (Fortune et al., 2005, Shcherbakova et al., 2003). Likewise, human Pol ϵ has a different error signature than human Pol δ (Schmitt et al., 2009, Korona et al., 2011). Moreover, yeast mutator Pol ϵ has error biases more similar to its human mutator counterpart (Agbor et al., 2013) than to mutator variants of the other two yeast replicases. That such biases leave their mark in the genomes of tumors with polymerase defects is suggested by a recent study of human tumors with mutations in the exonuclease activity of Pol ϵ (Shinbrot et al., 2014). Mutations generated by the polymerases can also mark genomes over evolutionary time (Reijns et al., 2015, Lujan et al., 2014). For example, in MMR-deficient *S. cerevisiae*, mutation biases suggest that around 70% of substitutions occur on the nascent lagging strand, resulting in the replacement of G and C with T (with C-to-T at approximately twice the rate of G-to-T). These results are sufficient to explain the A/T-rich *S. cerevisiae* genome and enrichments of T relative to A and G relative to C in regions known to be replicated as the nascent lagging strand.

Asymmetries in replication fidelity also depend on the relative concentrations of the four dNTPs. The dNTP concentrations are not equal in undamaged cells, with dGTP present at a lower concentration than dATP, dTTP, or dCTP (Traut, 1994, Nick McElhinny et al., 2010). The dNTP concentrations can also increase upon DNA damage, to promote repair and improve survival but at the cost of increasing the overall mutation rate (Chabes et al., 2003). The effects of nucleotide pool imbalances on replication in undamaged cells have been studied in *S. cerevisiae* strains bearing mutants of *RNR1*. Mutants that alter dNTP pools are mutagenic *in vivo* (Kumar et al., 2011), and increased dNTP concentrations result in increased, strand-symmetrical mutagenesis in otherwise wild type yeast (Buckland et al., 2014) as well as in MMR-deficient yeast cells. In contrast, imbalances that decrease at least one dNTP concentration below normal result in asymmetric mutagenesis. For example, an *rnr1-Q288A* mutation that elevates dATP and dGTP but reduces dCTP causes lagging strand-specific mutagenesis (Kumar et al., 2011). This may be due to leading strand mutations that trigger Pol ϵ -dependent checkpoint activation (Navas et al., 1995) to slow replication and allow time to repair those mutations.

Mismatch repair of replication errors

Eukaryotic MMR is initiated by either of two mismatch recognition complexes, MutS α (Msh2-Msh6, Figure 2, panel G) or MutS β (Msh2-Msh3). These complexes have partially overlapping specificities, with MutS α recognizing and binding to base-base and small (1 to 3 base) insertion and deletion (indel) mismatches, whereas MutS β recognizes and binds to indel mismatches of one to about 15 bases. After recognition, a second heterodimer composed of MutL α (yeast Mlh1-Pms1 or mammalian Mlh1-Pms2, Figure 2, panel H), MutL β (Mlh1-Mlh2) or MutL γ (Mlh1-Mlh3) interacts with one of the two MutS heterodimers to promote downstream steps. For MMR to correctly excise the mismatch in the nascent strand, a signal is needed to direct MMR to the nascent rather than the template DNA strand. One molecule that is critical for this signaling is PCNA (Georgescu et al., 2015, Pluciennik et al., 2010). Via well-known motifs in PCNA and its partners, PCNA associates with several proteins important for replication and MMR, including Pol δ , Fen1, Exo1 and the eukaryotic MutS and MutL heterodimers (reviewed (Moldovan et al., 2007)). The two faces of PCNA differ from one another, which allows replication factor C (RFC) to asymmetrically load PCNA onto double strand DNA proximal to a 3' primer terminus with the polymerase-binding face oriented toward the terminus (Mossi et al., 1997, Naktinis et al., 1996). Loaded in this way, PCNA can unambiguously identify the nascent strand and provide the correct directionality for MMR. Guided by MutS α , PCNA, RFC, and ATP, the endonucleolytic activity of MutL α then nicks the nascent DNA strand to license mismatch removal (Figure 2, panel I). The mismatch can be excised by a nuclease, such as exonuclease 1 (Exo1), or it can be removed after strand displacement, to allow new, correct DNA synthesis and ligation to complete MMR (Figure 2, panel J).

Readers interested in MMR mechanisms are encouraged to read recent reviews that describe in greater detail what is known, and what remains to be discovered, about this important process (Jiricny, 2013, Modrich, 2006, Kunkel and Erie, 2005, Boiteux and Jinks-Robertson, 2013). Here we simply note that MMR efficiency varies widely due to several factors, some of which introduce strand asymmetry. For example, a study of MMR of 8-oxo-guanine•adenine mismatches in budding yeast suggested that lagging strand MMR is more efficient than leading strand MMR (Pavlov et al., 2003). This preference could be due to abundant DNA ends and/or PCNA associated with synthesis of Okazaki fragments. This led to the predictions that Pol α errors, being closer to the 5'-ends of Okazaki fragment termini than Pol δ errors, might be more efficiently repaired than Pol δ errors (e.g., Figure 2, panel K), and that errors in the continuously replicated leading strand might be repaired less efficiently than those in the lagging strand. Both predictions are supported by studies of *EXO1*-deleted *S. cerevisiae* cells, in which MMR efficiency at specific loci is highest for a Pol α variant and lowest for a Pol ϵ variant, with the effect partially dependent on Exo1 digestion of the mismatch-containing patch (Hombauer et al., 2011, Liberti et al., 2013).

Exo1 digestion is not the only way, or even the predominant way, to remove mismatches. In fact, mismatches made by variants of Pol ϵ are often repaired by Msh2-dependent MMR as efficiently, and often more efficiently, than mismatches made by variants of Pols α and δ (Lujan et al., 2014, Lujan et al., 2012, St Charles et al., 2015). Thus, in addition to Exo1 digestion from the 5' DNA ends associated with Okazaki fragments, other means of

mismatch removal are available. Evidence has suggested mismatch-containing patch removal by the 3'-exonucleases of Pols δ and ϵ (Tran et al., 1999), by the Fen1 flap endonuclease (Liu et al., 2015), by Pol δ strand displacement synthesis followed by flap excision (Kadyrov et al., 2009), and removal initiated by nicks introduced by other means, such as RNase H2-dependent incision at ribonucleotides incorporated into the nascent leading strand by Pol ϵ (Figure 2, panel K (Lujan et al., 2013, Ghodgaonkar et al., 2013)).

Ribonucleotides are also asymmetrically incorporated into DNA during replication

Despite steric clashes with incoming 2'-hydroxyl groups (steric gating), rNTP exclusion is imperfect and cellular rNTP concentrations are much higher than dNTP concentrations (Traut, 1994, Chabes et al., 2003). As a consequence, ribonucleotides are incorporated during replication *in vivo* (Reijns et al., 2012, Nick McElhinny et al., 2010, Henninger and Pursell, 2014). Newly incorporated ribonucleotides can be proofread (Figure 3) by Pol ϵ (Williams et al., 2012) and Pol δ (Clausen et al., 2013), but only weakly relative to robust proofreading of single base mismatches. The incorporation of ribonucleotides by variant polymerases can be used to monitor the roles of Pols α , δ and ϵ in DNA replication. For example, the Pol ϵ mutator variant (M644G Pol ϵ in *S. cerevisiae*) readily incorporates ribonucleotides into DNA during synthesis *in vitro* and during DNA replication *in vivo* (Nick McElhinny et al., 2010). These ribonucleotides are found in genomic DNA isolated from budding and fission yeast cells lacking RNase H2, the enzyme that initiates RER (Sparks et al., 2012) (Figure 3), a robust pathway for the removal of genomic ribonucleotides. In such cells, ribonucleotides are preferentially present in the nascent leading strand (Reijns et al., 2015, Williams and Kunkel, 2014, Williams et al., 2012, Clausen et al., 2015, Koh et al., 2015, Daigaku et al., 2015). In comparison, these same studies found ribonucleotides preferentially in the nascent lagging strand in yeast strains with mutator variants of Pols α and δ . The identification of strand-specific ribonucleotides support the roles of these polymerases in leading and lagging strand replication inferred from the mutagenesis studies mentioned above.

Ribonucleotides in DNA can have both beneficial and detrimental consequences. On the beneficial side, two studies mentioned above (Ghodgaonkar et al., 2013, Lujan et al., 2013) indicate that ribonucleotides incorporated into DNA by Pol ϵ , but not those incorporated by Pols α or δ , may act as strand discrimination signals for repairing mismatches. Additionally, evidence indicates that the presence of two ribonucleotides in DNA at the imprint site are important for mating type switching in fission yeast (Vengrova and Dalgaard, 2006). On the detrimental side, a subset of ribonucleotides incorporated into DNA can be mutagenic. This can occur when topoisomerase 1 (Top1) cleaves the DNA backbone at the site of a ribonucleotide (Joyce, 1997). If the ribonucleotide has been incorporated into a short repetitive DNA sequence by the mutator variant of Pol ϵ , Top1 cleavage initiates genome instability in the form of genome integrity checkpoint activation, replication stress, and short deletion mutagenesis (Nick McElhinny et al., 2010, Kim et al., 2011). Similar effects are not observed for ribonucleotides incorporated into DNA by variants of Pols α and δ (Williams et al., 2015), again revealing the asymmetric consequences of the three replicases on genome stability. Ribonucleotides in DNA also lead to large forms of chromosomal rearrangements (examples shown Figure 3), including gross chromosomal rearrangements (GCRs), loss of

heterozygosity (LOH) and non-allelic homologous recombination (NAHR) (Reijns et al., 2012, Allen-Soltero et al., 2014). Readers interested in further details are encouraged to read recent reviews on the effects of ribonucleotides in DNA (Williams and Kunkel, 2014, Jinks-Robertson and Klein, 2015, Potenski and Klein, 2014).

Selectivity, proofreading and MMR balance leading and lagging strand fidelity

Measurements of mutation rates in *E. coli* that were performed many years ago led to the suggestion that MMR most efficiently corrects the mismatches generated at the highest rates during replication (Kramer et al., 1984, Dohet et al., 1985, Schaaper, 1993). This appears to generally be true in yeast, both for proofreading and MMR (Lujan et al., 2012, St Charles et al., 2015). This is shown in Figure 4, where lower apparent nucleotide selectivity during lagging strand synthesis is offset by more efficient proofreading and more efficient MMR. This makes sense from an evolutionary perspective. Presumably, the evolution of a new or improved repair pathway would increase individual fitness most by minimizing the most frequent and/or the most deleterious types of mutations. Although the effects of MMR defects in yeast are quantitatively different than proofreading defects (Lujan et al., 2015, St Charles et al., 2015), importantly, their target biases are both overlapping and complementary (St Charles et al., 2015). Thus, although nucleotide selectivity is apparently lower during lagging strand replication, the collective action of proofreading and MMR minimizes mutational asymmetry and balances mutation rates across the two DNA strands (Lujan et al., 2012).

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Figure 1.

A simplified view of the normal eukaryotic DNA replication fork. The MCM helicase (mini chromosome maintenance; grey hexameric ring) encircles the leading strand DNA template and drives the replication fork forward from the origin to unwind the two DNA template strands (grey wires). The single stranded regions created by unwinding are coated with RPA heterotrimers (replication protein A, grey spheres). MCM, Cdc45, and the GINS (go-ichi-ni-san) complex interact to form the CMG helicase. Top1 interacts with CMG to remove positive DNA supercoils that accumulate ahead of the replication fork. Top1 may also be important for removal of torsional stress in the newly replicated DNA. GINS interacts with the Dpb2 subunit of DNA polymerase ϵ (Pol ϵ ; blue shapes). Pol ϵ is posited to be the normal leading strand (blue wire) replicase. Pol ϵ is processive, and is stimulated by PCNA (proliferating cell nuclear antigen; yellow trimeric rings) and the CMG complex. Ctf4 (flat grey trimer) ties the CMG complex to the Pol1 catalytic subunit of Pol α (red shapes). Pol α is bound to primase (orange shapes) and interacts with the Pol32 subunit of Pol δ (green shapes). Primase initiates synthesis of lagging strand Okazaki fragments by synthesizing a short RNA primer (thick orange wires), which are then extended through synthesis of a short stretch of DNA by Pol α (red wires) and then for a longer stretch by Pol δ (green wires). The RFC (replication factor C) complex loads PCNA onto DNA via an RPA-stimulated mechanism. As the double-stranded DNA grows, RPA is displaced and nucleosomes (cubic grey clusters) are deposited. Pol δ synthesis continues until it encounters a nucleosome, displacing the 5' terminus of the previous Okazaki fragment. The displaced flap is excised and the resulting nick sealed by sequential action of FEN1 and ligase, with RNases H1 and H2 (not shown) removing the residual RNA primer, in a process called Okazaki fragment maturation (reviewed (Balakrishnan and Bambara, 2013)).

Figure 2.

Alternate repair pathways for replication errors. (A) A mismatch is generated in the polymerase active site of a replicative polymerase (Replicase; grey shapes). DNA strands are represented by colored wires and PCNA by yellow trimeric rings. The polymerase can either pause to allow proofreading of the mismatch (PR; B–F), or it can extend from the mismatch and rely on post-replication mismatch repair (MMR; G–K). (B) The mismatch partitions from the polymerase active site to the exonuclease active site (Exo). Subsequent steps depend in part on replicase and leading/lagging strand status. (C) Polymerase δ (Pol δ; green shapes) can proofread errors made by itself in *cis* or by another Pol δ in *trans*. (D) There is indirect evidence that Pol δ can also proofread Pol ε (blue shapes) errors in *trans*. (E) Pol ε can proofread its own errors in *cis* (Flood et al., 2015). (F) Pol α (red shapes) lacks proofreading ability, but its errors may be removed by extrinsic Pol δ PR (as shown) or via strand displacement and flap excision (as in Fig. 1). (G) Mismatches that are extended by the

replicase (or another polymerase) are subject to MMR. The pathway shown is the best characterized, with others (via MutS β , MutL β , MutL γ) varying in details. (H) MutS α (brown shapes) recognizes and binds the mismatch in a PCNA- and ATP-dependent manner. Mismatch binding causes the DNA to kink. (I) MutL α (purple shapes; likely more than the one shown) recognizes the bound MutS α , also in a PCNA-dependent manner. At least *in vitro*, both processes require a nick and repair occurs on the nicked strand. MutL α endonuclease (Endo) nicks the nascent (presumably already nicked) strand at least once. (J) The resulting DNA patch is either displaced by subsequent Pol δ synthesis and resolved like an Okazaki terminus (Fig. 1) or an exonuclease, most commonly Exo1, resects the patch before re-synthesis and ligation. The replication strand and erroneous polymerase affect the choice between these alternatives. (K) There are many probable strand discrimination signals. Among them, Okazaki fragment termini and Ribonucleotide Excision Repair (RER) nicks are specific or preferential to the lagging and leading strands, respectively.

Figure 3.

Minimizing genomic ribonucleotide incorporation. Arrow thickness indicates relative numbers of mismatches. Arrows indicate transactions that alter or remove genomic ribonucleotides. The processes that facilitate and result from such actions are indicated by labels next to and terminal to such arrows, respectively. Processes with negative (mutations, slow growth, etc.), high fidelity, and unknown consequences are color coded red, green, and grey, respectively. For definitions and detailed discussions on the effects of RNaseH2 (Allen-Soltero et al., 2014), Top1 (Huang et al., 2015, Williams et al., 2013), Hnt3 (Tumbale et al., 2014), Srs2 (Potenski et al., 2014), Exo1 (Potenski et al., 2014), Tdp1 (Sparks and Burgers, 2015), and Tpp1 (Sparks and Burgers, 2015), please see references in this legend and within the section entitled *Ribonucleotides are also asymmetrically incorporated into DNA during replication*.

Figure 4.

Minimizing and balancing leading and lagging strand mismatches. Arrow thickness indicates relative mismatch abundance in the lagging (green arrows) and leading strands (blue arrows). Arrows that deviate from the horizontal indicate mismatches that are repaired. The processes that facilitate and result from such removal are indicated by labels next to and terminal to such arrows, respectively. Mismatches that avoid repair lead to mutations or genome instability (red arrows). Because rates vary over five orders of magnitude visual magnifications are used where needed (circular magnifying lenses). Relative mismatch levels after each process are indicated as mismatch ratios, leading strand versus lagging strand (LEAD:LAG). The three primary determinants of replication fidelity are base selection, proofreading (PR) and mismatch repair (MMR). (A) Polymerase active sites are many millions of times more selective for properly base-paired nucleotides over mismatched nucleotides. The 15-fold excess of lagging strand mismatches was estimated by taking overall mutation rates and strand bias (70% lagging strand; see panel B) seen across the genomes of MMR-deficient *Saccharomyces cerevisiae* (Lujan et al., 2014) and extrapolating back using apparent PR correction factors from reporter assays in PR- and/or MMR-deficient *S. cerevisiae* (St Charles et al., 2015). Since extrinsic PR efficiencies and wild type strand biases have not been well characterized, precise strand-specific biases in the absence or presence of *both* PR and MMR are unknown. (B) The vast majority of mismatches that escape base selection in the polymerase active site are excised by the exonucleases of Pols δ and ϵ . Some Pol α errors are likely removed instead during the flap excision step of Okazaki fragment maturation and are not represented here. Lagging strand mismatches still outnumber those on the leading strand. (C) The vast majority of mismatches that escape exonucleolytic PR are removed via MMR. (D) The few remaining mismatches, roughly

balanced between strands, are copied in the next round of replication. Each results in a mutation in one daughter cell and its descendants. As in panel A, the 1.6-fold excess of leading strand mismatches was estimated by comparing overall mutation rates and strand bias in MMR-deficient *S. cerevisiae* genomes and apparent MMR correction factors from reporter assays (Lujan et al., 2014, St Charles et al., 2015).

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Table 1

Replicase subunits. *S. c.* = *Saccharomyces cerevisiae*. *H. s.* = *Homo sapiens*. Entries are colored by complex, as per Figure 1: primase orange, Pols α , δ , and ϵ red, green, and blue, respectively. B subunits are evolutionarily conserved, essential for viability, and involved in protein-protein interactions and regulation (reviewed (Johansson and Macneill, 2010)).

Complex	<i>S. c.</i> protein	<i>H. s.</i> protein	Role(s)
Pol α	Pol1	p180	polymerase
Pol α	Pol12	p70	B subunit
Primase	Pri1	p49	primase
Primase	Pri2	p58	regulatory subunit
Pol δ	Pol3	p125	polymerase, exonuclease
Pol δ	Pol31	p50	B subunit
Pol δ	Pol32	p66	PCNA-binding, Pol1-binding
Pol δ		p12	PCNA-binding, regulatory subunit
Pol ϵ	Pol2	p261	polymerase, exonuclease, Ctf4-binding
Pol ϵ	Dpb2	p59	B subunit, GINS-binding
Pol ϵ	Dpb3	p17	processivity factor
Pol ϵ	Dpb4	p12	processivity factor