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Reconsidering DNA Polymerases at the Replication Fork in Eukaryotes

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Abstract

The distribution of DNA polymerase activities at the eukaryotic DNA replication fork was "established," but recent genetic studies in this issue of *Molecular Cell* raise questions about which polymerases are copying the leading and lagging strand templates (Johnson et al, 2015).

"Everything is complicated. If it were not so, life and poetry and everything else would be a bore." Based on recent literature (Johnson et al, 2015), one could conclude that the molecular events at eukaryotic DNA replication forks, particularly how various DNA polymerases combine to copy both the leading and lagging stand templates, are far from boring, but indeed downright complicated.

Because the two strands of the DNA double helix have opposite polarity and all DNA polymerases replicate in the same direction (5′ to 3′), DNA replication occurs continuously on one strand, the leading strand, but discontinuously via short Okazaki fragments on the other strand, the lagging strand. The different strategies have consequences for the machineries that copy the strands, including which DNA polymerases are involved and also how DNA damage can be repaired.

This entire issue came to the fore when, in addition to DNA polymerases α and δ, a third "replicative" DNA polymerase, polymerase ε, was identified in the yeast *S. cerevisiae* and later found to be conserved in all eukaryotes (Johansson and Dixon, 2013). DNA polymerases α and δ are sufficient to replicate the Simian Virus 40 genome (Figure 1A), long thought of as a model for the eukaryotic DNA replication fork (Waga and Stillman, 1998). A role for DNA polymerase ε proved to be perplexing because the *POL2* gene encoding the largest subunit of the four-subunit DNA polymerase ε is essential, but its N-terminal DNA polymerase catalytic activity can be deleted and yeast are still viable. The essential activity actually lies within the Pol2 C-terminal domain that is involved in the intra-S phase detection of DNA damage and induction of checkpoint signaling to repair damage and maintain fork stability (Dua et al., 1999).

The assignment of DNA polymerases to specific strands during DNA replication in eukaryotic cells has been studied by using mutant versions of DNA polymerases δ and ε with specific error signatures (reviewed in Johansson and Dixon, 2013; Williams and

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Kunkel, 2014). The studies showed, apparently clearly, that polymerase ε replicated the leading strand and polymerase δ replicated the lagging strand (Figure 1B).

Recent biochemical studies have shown that DNA polymerases α and ε, but not δ, are necessary and sufficient for the initiation of DNA replication at origins of DNA replication (Yeeles et al., 2015), but these in vitro observations do not address the strand assignment for complete DNA replication in vivo. Other biochemical studies from the O'Donnell laboratory have reconstituted DNA replication of leading and lagging strands, assigning DNA polymerase ε for leading-strand synthesis and polymerase δ for lagging-strand synthesis (Georgescu et al., 2014, 2015). They even identified a mechanism that prevents polymerase δ from competing with polymerase ε on the leading strand. Moreover, the structure of polymerase ε shows that it can tightly clamp onto DNA even without PCNA, making it an excellent candidate for the leading-strand polymerase (Hogg et al., 2014). But PCNA may still be required on the leading stand to enable coupling of nucleosome assembly by CAF-1 and other PCNA-associated functions (Figure 1B). Moreover, polymerase ε is directly associated with the CMG (Cdc45-Mcm2-7-GINS) helicase that travels on the leading-strand template DNA (Johansson and Dixon, 2013). Thus, the distribution of labor for polymerases δ and ε makes biochemical sense. Indeed, polymerase ε is enriched on the leading strand and polymerase δ on the lagging strand in vivo (Yu et al., 2014), but an excess of DNA polymerase δ on the lagging would be expected even if polymerase δ replicated both strands since more polymerase molecules are required on the discontinuously synthesized lagging strand. Nevertheless, from genetic and biochemical analysis, it seemed very clear that polymerase ε primarily replicates the leading strand and polymerase δ the lagging strand.

However, the paper by Johnson et al. (2015) in this issue raises the entire question of strand assignments again and concludes that polymerase δ replicates both leading and lagging strands, just like the SV40 model (Figure 1C, normal mode). They attribute the different genetic results to the use of different strains of yeast and to different pathways for repair of misincorporated nucleotides on the leading versus the lagging strand.

Error correction on the leading and lagging strands is likely to be different since the mechanisms of DNA synthesis are different. Johnson et al. suggest that mismatch repair is different on the lagging strand compared to the leading strand— notably that the proofreading activity of DNA polymerase ε is redundant with the exonuclease Exo1 for error repair on the leading stand, but not on the lagging strand. They suggest that the different mismatch repair mechanisms on the leading and lagging strands, coupled with the strains employed can explain the different results.

Evidence that polymerase ε primarily replicates the leading strand also emerged from data showing that ribonucleotides (rNMPs) were preferentially incorporated into the leading strand during DNA replication, in both *S. cerevisiae* and *S. pombe* (reviewed in Jinks-Robertson and Klein, 2015). These data include very impressive whole-genome analyses of rNMP incorporation into the leading strand when a mutant polymerase ε that promiscuously inserted rNMPs into DNA was employed. Importantly, a strain containing an allele of polymerase ε that was more stringent in rNMP discrimination than the wild-type polymerase ε incorporated less rNMP into the leading strand than the strain with the error-prone

polymerase ε. In contrast, when error-prone polymerases α and polymerase δ were present, rNMP incorporation was detected in the lagging strand. So this data seems to strongly support the model shown in Figure 1B. But a third model has now been suggested, namely that polymerase δ normally replicates both strands of the DNA, but that occasionally a switch to polymerase ε on the leading strand can be induced by replication errors, thereby coupling checkpoint signaling to repair of the DNA damage (Figure 1C). This model may explain why mutations in the polymerase ε catalytic residues have a dominant negative effect, suggesting that this inactive polymerase gums up replication (Dua et al., 1999).

The experiments showing preferential incorporation of rNMPs experiments were done in strains lacking the RNase2 enzyme that normally nicks the DNA 5′ to the rNMP in the DNA, creating a 3′-OH that is preferentially extended by DNA polymerase δ, creating a flap for rNMP excision much like strand displacement mechanisms used on the lagging strand. The absence of RNase2 causes extensive replicative stress (reviewed by Williams and Kunkel, 2014), activating the DNA-damage response pathway involving the essential domain of the polymerase ε large subunit. It is therefore possible that in the absence of RNase2, when polymerase δ incorporates an rNMP during leading-strand replication, it stimulates an alternative rNMP repair pathway that involves switching to polymerase ε to remove the rNMP or repair topoisomerase 1 induced DNA damage (Figure 1C). Such a repair mechanism by polymerase ε would only work on the leading strand where it is physically located; thus, rNMPs would be incorporated into that strand during the repair process when an error prone polymerase ε is present. When an error-prone DNA polymerase δ strain is employed, such errors would be repaired by the wild-type polymerase ε, leaving little trace of rNMP on the leading strand. Consistent with this model, on the lagging strand, preferential rNMP incorporation would be detected only in strains with either an errorprone polymerase δ since polymerase ε does not operate on the lagging strand for DNA synthesis or repair. Thus, the data could be construed as supporting the model in Figure 1C where polymerase δ replicates both strands but polymerase ε preferentially ensures leading-strand fidelity.

If the model in Figure 1C is correct, then genetic stability on the leading strand and lagging strand would be different due to the different repair pathways employed. For example, the location of polymerase ε-associated checkpoint proteins such as Mrc1, Dpb11, and Drc1/Sld2 (Osborn et al., 2002) could preferentially signal DNA damage that occurs on the leading strand. DNA-damage-dependent polymerase switching could also promote programed switches in gene expression such as mating type gene in *S. pombe* (see Williams and Kunkel, 2014). Such imprinting is thought to be marked by an rNMP-containing gap in the leading-strand template DNA, and recognition of this gap by the replicative helicase or polymerase may trigger a switch to DNA polymerase ε-coupled recombination.

The new paper by Johnson et al. will generate much discussion, and the polymerase assignment debate will continue. But, importantly, all of the genetic studies dealing with this issue, including those of Johnson et al., employ mutant strains that inform what is going on in the mutant condition (including all genetic variation in the strains used), suggesting caution about interpreting what is really going on in wild-type cells.

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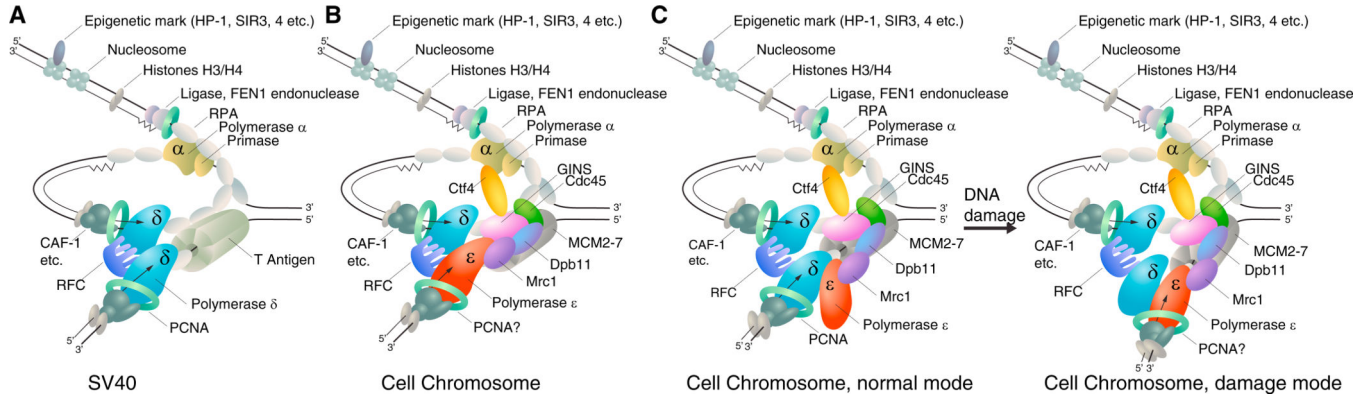

Figure 1. DNA Polymerases at the Eukaryotic DNA Replication Fork

(A) DNA polymerase δ synthesizes DNA during lagging (discontinuously synthesized, top) and leading (continuously synthesized, bottom) replication.

(B) The prevailing model in which DNA polymerase δ synthesizes the lagging strand and polymerase ε the leading strand.

(C) A potential new model in which DNA polymerase δ normally replicates both strands and, upon DNA damage in the leading strand template, a switch to polymerase ε occurs, linking DNA-damage detection to the essential role for polymerase ε and associated checkpoint proteins. In all cases, DNA polymerase α is coupled with primase to synthesize a RNA-DNA primer on the lagging strand that is recognized by RFC and PCNA to switch to the replicative polymerase. PCNA couples other events at the replication fork, such as nucleosome assembly.