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Who is leading the replication fork, Pol ε or Pol δ?

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Several studies in the past decade support a model wherein DNA polymerase ε (Pol ε) carries out the majority of leading strand DNA replication of the undamaged eukaryotic nuclear genome. Now a new paper in Molecular Cell from the Prakash laboratory challenges this model, claiming instead that Pol δ is the major replicase for both strands, and that Pol ε's primary role is only to proofread errors made by Pol δ during leading strand replication (Johnson et al., 2015). While we fully subscribe to the idea that the replication fork is plastic and that its composition can adapt to various challenges, we believe the foundation for an unchallenged replication fork remains as established before the Prakash paper, for the following reasons.

Mutation rate data

Our studies with mutants of the *POL3* and *POL2* genes encoding the catalytic subunits of Pol δ and Pol ε, respectively, revealed that they have two unique properties that lend themselves to studying strand-specific incorporation during replication. The first is that they are asymmetric mutators. As but one example, for the two mispairs that lead to AT→GC transitions, budding yeast Pol δ containing a L612M mutation in the polymerase active site misincorporates dGMP opposite template T much more frequently than dCMP opposite template A. When we eliminated error correction by deleting the mismatch repair gene *MSH2* (Lujan et al., 2014; Nick McElhinny et al., 2008), the *msh2Δ pol3-L612M* strain exhibited a large synergistic increase in AT→GC transitions compared to the wild type and *msh2Δ* strains, and relative to known replication origins, these mutations occurred in a pattern consistent with a model wherein Pol δ's primary role is in lagging strand replication. Importantly, the synergistic increase was very strong, providing confidence that the large majority of the mutations in the *msh2Δ pol3-L612M* strain were indeed due to the Pol δ-L612M change. However, strong mutators rapidly accumulate suppressor mutations that result in wide variations in mutation rates in the mitotic progeny of double mutants. This was obvious in our study of the *msh2Δ pol3-L612M* mutant (see Figure S1 in (Nick McElhinny et al., 2008)), leading us to quantify mutation rates in cultures obtained by limited outgrowth of haploid spores germinated from meiotic progeny of the heterozygous diploids *pol3-L612M/pol3-L612M MSH2/msh2Δ*. This was not done in the Prakash study.

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Instead, they obtained double-mutant strains by two subsequent transformations involving several rounds of outgrowth from single cells, and obtained mutation rates that were only a few percent of the high rates we determined. Thus, whether from accumulating suppressor mutations during extensive propagation or for other reasons, their strains lack the high mutation rates we used to assign mutations specifically to Pol δ-L612M errors. In addition, their mutational spectrum for one orientation of *URA3* in the S288c background shows strong GC→TA hotspots at base pairs 679 and 706 in *URA3* that together constitute a third of the observed mutations. The authors suggest that those substitutions preferentially originated from template G-dAMP mismatches. However, those two hotspots are missing in a strain having *URA3* in the opposite orientation. This would lead to the paradoxical suggestion that Pol δ-L612M does not replicate the lagging strain, yet neither they nor we imply that this is the case. Instead, our data suggest that the majority of GCTA mutations in undamaged cells actually result from the complementary C-dTMP mismatch (Lujan et al., 2014; Nick McElhinny et al., 2008). Therefore, we conclude that those two mutation hotspots, and by extension other hotspots, support, rather than disprove, our favored model.

Asymmetric ribonucleotide incorporation

A second useful feature of Pol δ and Pol ε variants is that they increase the incorporation of ribonucleotides into DNA. This occurs *in vitro* and in yeast in which newly incorporated ribonucleotides remain in the genomes of RNase H2 deficient (*rnh201Δ*) strains that are defective for ribonucleotide excision repair. Mapping data using next-generation sequencing shows increased ribonucleotide incorporation into the nascent lagging strand for *pol3-L612M/G* budding yeast variants and for an equivalent fission yeast variant, and increased ribonucleotide incorporation into the nascent leading strand of the *pol2-M644G* variant and its fission yeast equivalent (Clausen et al., 2015; Daigaku et al., 2015; Koh et al., 2015; Reijns et al., 2015). The straightforward explanation of these results is that Pol ε primarily replicates the leading strand and Pol δ primarily replicates the lagging strand. The Prakash study offers the alternative explanation that Pol ε does not incorporate ribonucleotides during replication, but rather only proofreads ribonucleotides incorporated by *pol3-L612M*, and does so only during leading strand replication. However, Pol δ itself has little or no ability to proofread DNA termini containing ribonucleotides, but it can extend them, so there is no reason for Pol δ to dissociate to allow Pol ε to gain access to these termini. Moreover, while Pol ε can intrinsically proofread its own mistakes, current evidence suggests that it has little or no ability to extrinsically proofread mistakes made by Pol δ (Flood et al., 2015). Finally, if Pol ε was important only for proofreading ribonucleotides incorporated by Pol δ into the nascent leading strand, then in an *rnh201Δ* strain with wild-type polymerases, the ribonucleotide density in the nascent leading strand should be lower than in the nascent lagging strand, whereas it is actually higher (Clausen et al., 2015). These facts do not fit a model in which Pol δ is normally the primary leading strand replicase. However, they do fit a model wherein Pol ε as the major leading strand replicase, and this role is supported by elegant DNA replication studies *in vitro* (e.g., see (Georgescu et al., 2014)). As discussed (Kunkel and Burgers, 2008), our favored model wherein Pol ε is the primary leading strand replicase does not exclude an important role for Pol δ in leading strand replication in certain regions of the undamaged genome and/or when the genome is under stress.

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