The evolving tale of Pol2 function

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DNA replication is complex and highly regulated, and DNA replication errors can lead to human diseases such as cancer. DNA polymerase ε (polε) is a key player in DNA replication and contains a large subunit called POLE, which possesses both a DNA polymerase domain and a 3′–5′ exonuclease domain (EXO). Mutations at the EXO domain and other missense mutations on POLE with unknown significance have been detected in a variety of human cancers. Based on cancer genome databases, Meng and colleagues (pp. 74–79) previously identified several missense mutations in POPS (pol2 family-specific catalytic core peripheral subdomain), and mutations at the conserved residues of yeast Pol2 (*pol2-REL)* showed reduced DNA synthesis and growth. In this issue of *Genes & Development*, Meng and colleagues (pp. 74–79) found unexpectedly that mutations at the EXO domain rescue the growth defects of *pol2-REL*. They further discovered that EXO-mediated polymerase backtracking impedes forward movement of the enzyme when POPS is defective, revealing a novel interplay between the EXO domain and POPS of Pol2 for efficient DNA synthesis. Additional molecular insight into this interplay will likely inform the impact of cancer-associated mutations found in both the EXO domain and POPS on tumorigenesis and uncover future novel therapeutic strategies.

Faithful genome replication is essential for the maintenance and inheritance of genetic information and the well-being of organisms. In humans, errors in DNA replication can fuel the development of genome instability syndromes, premature aging, and tumorigenesis. Therefore, despite decades of study, there remains great interest in better understanding the complex process of DNA replication and its regulation (Stillman 2022).

DNA replication in eukaryotic cells initiates from large numbers of sites throughout the genome called replication origins (Costa and Diffley 2022) and requires three well-conserved polymerases to complete the synthesis. These replicative polymerases, including the DNA polymerase α (polα), polδ, and polε, along with the replicative helicase and a host of other factors, form the replisome that synthesizes DNA at replication forks (Garg and Burgers 2005; Johnson and O'Donnell 2005; Pursell et al. 2007). The strand that is synthesized in the same direction as that of the moving replication fork (leading strand) is replicated continuously using primarily polε, whereas the strand synthesized in the opposite direction (lagging strand) is replicated discontinuously using primarily polδ (Burgers and Kunkel 2017). POLE is the large subunit of polε and supplies its catalytic activity in DNA synthesis. In addition to the catalytic domain, POLE also contains a 3′–5′ exonuclease domain (EXO), which is critical for correcting misincorporated nucleotides during DNA replication. Not surprisingly, mutations in POLE, in particular at its EXO nuclease domain, have been found in a variety of human cancers (Barbari and Shcherbakova 2017), making it an attractive target for further mechanistic studies.

The budding yeast *Saccharomyces cerevisiae* has been an excellent model system for the study of DNA replication. Advanced molecular techniques using yeast have greatly accelerated the identification of yeast replication factors and their homologs in other species (including humans) (Bell and Dutta 2002). Moreover, as one of the most genetically tractable and versatile model organisms, yeast provides great value for determining the biological effects of genetic variants of replication factors found in diseases such as human cancers (Cervelli et al. 2020). By taking advantage of these properties, Meng et al. (2020) used budding yeast as a model to characterize the impact of cancer-associated POLE mutations on DNA replication and genome stability, an exciting step toward understanding the many cancer variants of unknown significance (VUS) in POLE.

Despite its importance in DNA replication, only a few domains of Pol2, such as its P domain, had been studied in detail (Hogg et al. 2014). Meng et al. (2020) recently characterized a domain called POPS (pol2 family-specific catalytic core peripheral subdomain), which shows 71% sequence homology between the yeast Pol2 and the human POLE but is not found in other types of DNA

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polymerases. Furthermore, by searching cancer genome databases, the investigators identified several missense mutations at this domain of the human POLE. However, the functional impacts of these mutations were not known. Meng et al. (2020) introduced three of these POPS mutations into the yeast Pol2, generating the *pol2-REL* allele, which contains mutations at three residues: R567C, E611K, and L621F. They found that *pol2-REL* cells unsurprisingly had reduced DNA synthesis in vivo. However, unlike previously characterized polε cancer variants at the exonuclease domain that induce hypermutations, *pol2-REL* caused genome hyperrearrangement with normal mutation rates (Meng et al. 2020). Further understanding of how this newly characterized POPS contributes to Pol2-mediated replication elongation was elusive until the more recent work of Meng et al. (2023).

In their study, Meng et al. (2023) sought to answer the above question by finding suppressors of the *pol2-REL* allele, mutating other Pol2 regions to rescue *pol2-REL* growth defects. They found that mutating two catalytic residues of the exonuclease domain of Pol2 (*EXOcd*) improved *pol2-REL* growth, suggesting a functional interplay between POPS and the EXO domain. In contrast, removal of the Exo1 protein, an exonuclease involved in DNA replication and DNA repair, was not able to improve *pol2-REL* growth or influence *pol2-EXOcd-REL* cells, showing a unique ability of the EXO domain mutation in restoration of the *pol2-REL* phenotype. The investigators went on to show that in addition to better growth, *pol2-EXOcd-REL* cells exhibited reduced levels of chromosomal rearrangements compared with *pol2-REL* cells, suggesting enhanced genome replication and stability. At the cell cycle level, DNA synthesis appeared to be faster in *pol2-EXOcd-REL* cells compared with *pol2-REL* cells, which did not appear to be due to a lack of replication-promoting factor association with the replicative helicase based on their protein–protein interaction data.

To assess whether the slowed growth of the POPS mutant is truly due in part to Pol2's exonuclease activity impairing replisome movement, Meng et al. (2023) used Replicon-seq to generate high-resolution genome-wide maps to visualize the movement of replication forks. The map for *pol2-EXOcd-REL* was closer to that of the wild type than that of *pol2-REL* across the genome. The Replicon-seq data also showed an increase in the relative length of the leading strand in *pol2-EXOcd-REL* cells compared with *pol2-REL* cells. Based on these data, Meng et al. (2023) proposed an intriguing yin and yang model eloquently depicted in Figure 5 of their report, wherein EXO-mediated polymerase backtracking impedes the forward movement of the enzyme when POPS is defective.

These new findings advanced our understanding of Pol2 function and are undoubtedly exciting. However, with each answer comes more questions. How exactly does POPS counteract the role of EXO to allow efficient DNA replication? Meng et al. (2023) postulate that POPS may favor a conformation of Pol2 needed for strand synthesis and hinder the primer transfer to the EXO domain. Given that these mutations in POPS correspond to recurrent human cancer-associated mutations in POLE, further testing of this hypothesis and other possibilities may inform the impact of these mutations on tumorigenesis and uncover novel means to counteract it. We look forward to continued insights into this new POPS and EXO antagonism and its potential effects on human cancers.

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