Breakthrough for a DNA break-preventer

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Mammalian genomes encode about 16 distinct DNA polymerases that participate in different aspects of DNA replication, DNA repair, recombination, or bypass of DNA damage (1, 2). The DNA polymerases used for normal replication generally cannot proceed on damaged DNA. When a damaged site is encountered, replication is stalled at least temporarily, and the lesion may be bypassed by invoking a process of translesion DNA synthesis (TLS) mediated by specialized DNA polymerases, or by switching to another undamaged DNA template. In a report in PNAS (3), Lee et al. significantly advance the possibilities for understanding this process by their work on DNA polymerase ζ (Pol ζ), arguably the most important of the specialized DNA polymerases. The authors describe the purification of an active form of the human Pol ζ holoenzyme composed of four subunits, which opens up the possibility for detailed biochemical and structural studies of this essential enzyme. Pol ζ from the yeast *Saccharomyces cerevisiae* has been available, but the mammalian

protein has additional functions (for example, it is essential in mammals, but not in yeast), and the catalytic subunit (called REV3L) is about twice the size of the yeast protein.

Pol ζ is exceptionally significant in the defense of mammalian cells against DNA damage. Pol ζ is needed for the bypass of many DNA lesions by TLS, although it can be mutagenic if an incorrect base is inserted opposite a mis-instructional lesion in the DNA template (4–6). However, a perilous consequence of delaying bypass is that the machinery at the DNA replication fork can collapse, leaving it exposed to enzymes in the cell that cut the DNA and form a double-strand break. Pol ζ aids in replication of some naturally occurring DNA sequences that are inherently difficult to traverse, such as the “fragile-site” regions in mammalian genomes, or sequences forming non-B DNA structures (7, 8).

The biological function of Pol ζ in mammals has been challenging to unravel because disruption of *Rev3L* is incompatible with

mouse viability (6). The use of conditional gene-deletion models has allowed the study of Pol ζ function in mice and cells. Immune system T-cells do not normally survive following *Rev3L* deletion, but in a p53 mutant background, some cells escape checkpoint controls and can form lymphomas (9). In fact, loss of Pol ζ function is selectively favored for tumorigenesis in this setting. Specific deletion of *Rev3L* from epithelial tissues leads to very high skin sensitivity to UV radiation (10). If such mice are not challenged with UV radiation but simply left to age, they developed tumors in *Rev3L*-deleting epithelial tissues, especially in specialized sebaceous glands. This tumor formation is consistent with the chromosomal instability that accompanies the Pol ζ defect (9, 10). At the same time, Pol ζ is a potentially important target in cancer therapy, because in mouse xenograft models suppression of REV3L function can sensitize intrinsically resistant tumors to chemotherapy and reduce the frequency of acquired drug resistance of relapsed tumors (11).

Lee et al. (3) coexpressed human REV3L and a known protein partner, MAD2L2 (also known as REV7) in mammalian cells in culture. The authors initially encountered very low expression and heterogeneity in their preparations, and solved these problems by systematically deleting various segments of human REV3L in its central domain (Fig. 1A). Active Pol ζ was purified with the aid of a tag on REV7. Two additional cellular components were detected after analysis by mass spectrometry; these were the mammalian POLD2 and POLD3 proteins. This finding dovetails with recent discoveries that POLD2 and POLD3, previously known as subunits of the replicative DNA polymerase δ , are indeed subunits of Pol ζ . An iron-sulfur cluster near the C terminus of REV3L provides a docking site for POLD2 (12), and POLD3 associates with POLD2. In the yeast *S. cerevisiae* the orthologous Pol δ subunits are designated *pol31* and *pol32* and they associate with both Pol δ and *Rev3* via a similar

Fig. 1. (A) Diagram of the full-length human Pol ζ catalytic subunit (REV3L) with domains noted; Lee et al. (3) modified the *REV3L* gene to make the shorter protein REV3L 4-2, the optimal version for purification that also retained significant polymerase activity. Accessory subunits MAD2L2 (REV7), POLD2, and POLD3 are shown binding to the appropriate locations on the REV3L 4-2 protein. (B) Multiple polymerase model for bypass of a DNA lesion: (i) Pol δ (including subunits POLD1, POLD2, and POLD3) stalls at a DNA lesion; (ii) Pol η [which can bind to PCNA simultaneously with Pol δ (2)] engages the template and incorporates one to two nucleotides; (iii) Pol η is removed, POLD1 disengages from POLD2, and is replaced by the Pol ζ (REV3L and REV7) subunits, which add several nucleotides until the DNA structure normalizes; (iv) POLD1 replaces Pol ζ and continues with DNA replication.

Author contributions: R.D.W. and S.S.L. wrote the paper.

The authors declare no conflict of interest.

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4Fe-4S cluster that is conserved in B family DNA polymerases (13, 14). The shared association of the catalytic subunits of Pol δ and Pol ζ with these additional subunits may provide a mechanism for the two polymerases to switch places when normal DNA replication is stalled at a template DNA lesion (12).

Almost all previous studies with purified yeast Pol ζ have been performed with the two-subunit version (Rev3 and Rev7 only). The two-subunit form of the enzyme has low fidelity in copying nondamaged DNA, although not as low as other TLS DNA polymerases (15). Pol ζ does make more complex mutations at short repeated sequences capable of forming hairpin structures (8). Kinetic measurements will be needed to firmly establish the discrimination of the four-subunit form between correct and incorrect nucleotides. The POLD3 subunit interacts with the DNA polymerase clamp ring, called proliferating cell nuclear antigen (PCNA), which may also influence the processivity and fidelity of the four-subunit form. POLD3 was recently identified as a gene product that helps ameliorate the consequences of DNA breaks formed under replication stress conditions (16). This might be related to POLD3's role as a subunit of Pol δ , but now the potential role of Pol ζ in preventing break formation under stress conditions should be considered. In fact, it is known that without Pol ζ , DNA double-strand breaks form in normally replicating cells, with ensuing chromosomal rearrangements (10, 17–19).

Genetic experiments suggest that two specialized DNA polymerases are needed for bypass of some lesions, with one polymerase inserting a nucleotide opposite a lesion and

the second extending the “mismatched” DNA primer to accomplish successful translesion synthesis (Fig. 1B). Such a reaction has been

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demonstrated in a mixed species system with two-subunit yeast Pol ζ , but Lee et al. (3) here show that human Pol η and human four-subunit Pol ζ can cooperate to fully bypass an adduct caused by the drug cisplatin.

Only the C-terminal 25% of the large REV3L protein contains the residues conserved in B-family DNA polymerases. The Lee et al. (3) study shows that some segments of the protein are needed for activity, and some are not. It will be of interest to determine the functions of both types of regions, particularly as some of those that are unnecessary for polymerase activity appear to be evolutionarily conserved as assessed by protein alignments. An overall view of the four-subunit yeast Pol ζ has recently been obtained by electron microscopy (20). The work of Lee et al. (3) should accelerate progress toward a 3D structure of human Pol ζ , which will contain additional structural regions.

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