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How to accurately bypass damage

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Abstract

Ultraviolet radiation can cause cancer through DNA damage — specifically, by linking adjacent thymine bases. Crystal structures show how the enzyme DNA polymerase η accurately bypasses such lesions, offering protection.

In this issue, two papers — by Silverstein *et al.*¹ (page 1039) and Biertümpfel *et al.*² (page 1044) — describe the crystal structure of the enzyme DNA polymerase η (Pol η), which can efficiently and accurately overcome DNA damage caused by ultraviolet radiation. These data are of particular interest because they elucidate how the inactivation of Pol η leads to XPV, a variant form of xeroderma pigmentosum — a type of severe skin cancer in humans.

DNA polymerase enzymes mediate DNA replication and repair. In eukaryotes (organisms such as animals, plants and yeast), at least 14 such polymerases, with diverse functions, have been identified³. For instance, DNA polymerases of the Y family specialize in DNA-lesion bypass. According to the polymerase-switch model⁴, when a high-fidelity replicative polymerase encounters a DNA-distorting lesion, it stalls and is replaced by one or more lesion-bypass polymerases. The bypass polymerases transit the lesion and extend the DNA until the perturbation has been passed. The replicative polymerase then resumes its task of rapidly synthesizing the growing DNA chain.

Humans have four Y-family DNA polymerases: Pol ι , Pol κ , REV1 and Pol η . The crystal structures of ternary complexes — containing one of these polymerases, together with template and primer DNA, and the deoxyribo-nucleoside 5'-triphosphate (dNTP) positioned ready for addition to the growing primer chain — have been solved for Pol ι (ref. 5), Pol κ (ref. 6) and REV1 (ref. 7). Together with the structures of the non-human Y-family polymerases Dpo4 (ref. 8) and Dbh (ref. 9), these structures have revealed that some features are unique to a particular Y-family member, whereas others are universal to all members.

For example, like other polymerases, all Y-family polymerases have a hand-like shape, with palm, fingers and thumb domains¹⁰, as well as an active site with evolutionarily conserved carboxylate-containing amino-acid residues (aspartic acid and glutamic acid) and two divalent metal ions, usually magnesium ions (Mg²⁺). In addition, being generally of low fidelity, Y-family polymerases have a spacious and solvent-accessible active site to facilitate

lesion bypass¹¹. This feature is in contrast to that of high-fidelity polymerases, the fingers of which close tightly on the nascent base pair to promote accurate replication^{10,12}. What's more, Y-family polymerases have a domain termed the little finger, or PAD, which aids in gripping the DNA^{11,13}. None of the human Y-family polymerases was found to be highly specialized for the error-free bypass of specific types of DNA damage, although the structures and biochemical evidence provided clues to the nature of the lesions that the enzymes are designed to process^{5-7,11,13,14}.

The most elusive and intriguing of the human bypass polymerases is Pol η . On a functional level, it is essential for error-free bypass of a highly prevalent lesion that results from exposure to ultraviolet radiation, including sunlight^{11,13}. The lesion is called a *cis-syn* thymine dimer (Fig. 1a, overleaf): two adjacent thymine bases on the same strand form two covalent bonds to produce an open-book-like structure, causing a distortion when incorporated in B-DNA — the most common conformation of DNA. Pol η bypasses this lesion in an error-free manner, correctly incorporating an adenine base opposite each of the thymines in the covalently linked dimer^{15,16}. How it can do this has remained a structural mystery.

Silverstein *et al.*¹ report two crystal structures of a yeast Pol η in ternary complexes: one with incoming dATP and a DNA template strand that contains two normal thymines, and another with dATP and a template strand that contains a *cis-syn* thymine dimer. Biertümpfel *et al.*² present a similar structural pair for human Pol η (Fig. 1b), together with additional structures showing the thymine dimer after replication has progressed so that two extra nucleotides have been incorporated (Fig. 1c). Together, the structures^{1,2} show that key structural elements are conserved across the evolutionary span from yeast to humans. To solve these structures, clever mutational strategies were required to break crystal-packing forces that came into play in an earlier study¹⁷.

The structures provide an elegant explanation for how the dimer is replicated in an error-free manner and for the curious fact that the dimer is as accurately replicated as unmodified thymines. Together with kinetic studies², they reveal how extension past the lesion can be efficiently carried out. What is the trick? Compared with other Y-family human polymerases, Pol η has a more spacious active site because the polymerase core (palm and fingers) is rotated away from the little finger, allowing two nucleotides to be housed in the active site instead of just one. This is necessary because the two thymines that are linked as a result of ultraviolet-radiation-induced damage cannot be uncoupled. Moreover, van der Waals forces and hydrogen-bonding interactions specifically hold the coupled thymines so that they can be partnered with adenine, hence the higher fidelity for the lesion than for the undamaged thymine.

Another feature that is unique to Pol η , accounting for the ready extension past the dimer, is the presence of a stiff spine, or 'molecular splint', that holds the growing duplex chain rigidly in its normal B-DNA conformation, without allowing slippage or other structural anomalies. In this respect, Pol η differs from other Y-family polymerases, all of which have a gap between the core and the little finger instead of the continuous protein surface that creates the molecular splint. The open space, the volume of which differs among

polymerases, allows greater flexibility and opportunity for error in the growing duplex. Gratifyingly, mutations that are observed in patients with XPV are explained by the structure of the human enzyme. For example, the arginine residue present at position 111 of Pol η is part of the molecular splint, and mutation of the bases encoding this amino acid to produce histidine has been observed in patients with XPV and is likely to disrupt the splint.

Every open door leads to another door, and the same holds true for these fascinating Pol η structures. Resolving the structures of binary complexes — without the dNTP — is essential to gain insight into conformational changes that may be induced by the binding of the dNTP. So far, all evidence suggests that the large conformational movement, that is, the closing of the fingers on dNTP binding, seen in high-fidelity polymerases does not occur in members of the Y family. But there are indications of more subtle conformational reorganizations, the details of which may depend on the specific bypass polymerase. These early movements need to be understood in order to delineate the mechanistic details of the ensuing chemical reaction of nucleotide addition.

The suggestions that Y-family polymerases in general are engaged in other forms of DNA repair besides lesion bypass are intriguing. For example, Pol κ has recently been implicated in nucleotide-excision-repair synthesis¹⁸. Pol η has also been implicated in the repair of DNA strand breaks, specifically in filling in gaps that are created by the displaced loops produced in homologous recombination^{19,20}. And Biertümpfel and colleagues' structure suggests that another DNA-binding site on the other side of the enzyme may be used in this process².

It will certainly be exciting to determine how Pol η is involved in these repair processes and in other cellular processes such as the generation of antibody diversity. Polymerase structure and function remains a research frontier despite the amazing progress that has been achieved in the past decade.

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Figure 1. Accurate lesion bypass by Polη

a, Ultraviolet (UV) radiation catalyses covalent linkages between two adjacent thymine (T) bases in a DNA strand so that they form a distorting mutagenic lesion, the T-T dimer. Backbone phosphorus, yellow; oxygen, red; nitrogen, dark blue. **b**, Biertümpfel *et al.*² report the structure of human Polη in a ternary complex containing dATP correctly positioned opposite the 3' base of the thymine dimer in the template strand. This structure reveals an active site (the area within the dashed lines) that is enlarged compared with other Y-family polymerases and that accommodates the two covalently linked thymine bases to permit

‘Watson–Crick’ hydrogen bonding with the dATP, which is oriented for catalysis mediated by two metal ions (purple spheres). Silverstein and colleagues¹ present a related ternary complex of yeast Pol η , uncovering a remarkably similar strategy for thymine-dimer bypass (not shown). Thymine dimer, orange; template bases, blue; primer bases, green; incoming nucleotide, white. **c**, The crystal structure of human Pol η is shown in a ternary complex² in which the DNA has been replicated past the thymine-dimer lesion by the addition of two nucleotides (A). Unlike other lesion-bypass polymerases, Pol η contains a molecular splint — a continuous protein interface between the core (palm and finger) and little finger domains, which cannot be seen in this view — that holds the growing duplex in its normal B-DNA conformation, allowing efficient and accurate extension past the thymine dimer.