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Unfaithful DNA polymerase caught in the act

Jiricny, J

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Sean M. Burgess
Section of Molecular and Cellular Biology
Center of Genetics and Development
University of California, Davis
Davis, California 95616

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Unfaithful DNA Polymerase Caught in the Act

The 3D structures of all 12 mispairs formed in the active site of a DNA polymerase (Johnson and Beese, 2004) help explain their differential effects on polymerase stalling and on translocation of the primer terminus to the enzyme's proofreading site.

One of the key dogmas of molecular biology is that the nucleotide sequence of genomic DNA has to remain unchanged during the lifetime of a cell. This requires that the main processes of DNA metabolism, replication, transcription, and recombination do not bring about changes in the DNA sequence. This is easier said then done, however, given that each time the two strands of a duplex are separated, cytosines can deaminate to uracils (U) or guanines oxidize to 8-oxoguanines (GO), modifications that, left uncorrected, would result in C→T (transition) or G→T (transversion) mutations during DNA replication. These mutations arise because the replicating DNA polymerase pairs the modified bases in the template strand with "best-fit" partners from the dNTP pool, in both these cases adenines. Although the resulting U•A and GO•A base pairs allow the polymerase to continue on its quest to duplicate the genome, the sequence of the newly synthesized strand is altered. But mutations can arise during replication also in the absence of base modifications. Thus, although DNA polymerases are very exact machines, they misincorporate a noncomplementary nucleotide once in every 10,000–1,000,000 (Kunkel, 2004). Such accuracy, although enviable, would introduce at least 1000 errors into the human genome each time the cell divides. Given that mutations are associated with genetic diseases and cancer, this error rate is clearly unacceptable. Fortunately, the accuracy of DNA replication is further enhanced by proofreading and by mismatch repair. The former activity is dependent on the fact that DNA polymerases have difficulties in extending from a mismatch (Benkovic and Cameron, 1995). The misaligned terminus of the growing (primer) strand, hitherto located in the active site of the stalled polymerase, can thus be translocated to another site, where a 3′→5′ exonuclease removes several residues from the 3′ end, starting with the noncomplementary one. This shorter, but error-free, primer can then reanneal to the template, and DNA synthesis can resume. The proofreading exonuclease function is extremely important for genomic stability, as it generally improves the fidelity of DNA synthesis by two to three orders of magnitude. Indeed, the *mutD5* mutant of *E. coli*, in which the 3′→5′ exonuclease subunit of the DNA polymerase III is defective, is one of the strongest mutator strains known (Schaaper and Radman, 1989). Despite the clear biological importance of this function, however, our understanding of the molecular criteria that determine whether the primer end will be extended or translocated to the proofreading site is limited. Studies of extension kinetics from primers with mispaired 3′ termini (Creighton and Goodman, 1995) have provided us with a list of those that do and those that don't, but little more. In the March 19th issue of *Cell*, Johnson and Beese offer us an insight into the structural basis underlying the kinetic data by describing the 3D structures of 12 mispaired primer/template combinations, situated within the active site of *Bacillus* DNA polymerase (Johnson and Beese, 2004). What makes this study notable is that 10 of the 12 mispaired termini were generated in situ, by soaking one of the three incorrect dNTPs into the protein/DNA cocrystals and letting the polymerase extend the primer in the crystal by this one nucleotide—and all this took was to substitute Mg^{2+} for Mn^{2+}. Incredibly, polymerase could even extend from some of the mismatches formed in the crystal!

The structures of the various mispairs could be sorted into four distinct groups: (1) those that bring about primarily distortions of the template strand, (2) those that disrupt the primer stand and the catalytic site of the enzyme, (3) those that distort both primer and template strands, and (4) those that fray at the insertion site. Interestingly, these four groups did not necessarily overlap with the mismatch class. Thus, group 1 contains G•T, A•C (purine•pyrimidine), G•G (purine•purine), and T•C (pyrimidine•pyrimidine) mispairs; group 3 includes A•G and T•G; and group 4 A•A, G•A, and C•C. These structures hindered the polymerase from extending the mispaired primer to different extents. The A•G, T•T, T•G,

and C•C lesions could not be extended in the crystal, which implies that they would most likely be readily translocated to the proofreading site. In contrast, the G•T, C•T, and G•G could be extended. Unbelievably, the G primer could be extended right to the end of the T template, which involved the stepwise addition of five nucleotides. Structural analysis of the intermediate extension products revealed that the polymerase might sense the presence of the G•T mispair even after it left the active site, as the primer terminus was distorted even when the mispair moved three residues into the duplex binding site of the enzyme. Interestingly, the bases in this mismatch no longer appeared to be in the wobble conformation. Instead, their geometry resembled that of a G•C pair, which implied that one tautomerized to the enol form. Tautomeric shift of bases was predicted to account for the generation of mutations during DNA replication nearly 30 years ago (Topal and Fresco, 1976), but experimental evidence repeatedly pointed to the wobble base pair as the culprit. The present study shows that the latter structure is indeed formed in the polymerase active site, but it also demonstrates that the protein can trap the bases in unfavored tautomeric states when need be.

In spite of the fact that DNA polymerases can be forced to make mispairs and even extend from them in vitro, this happens rather seldom in vivo. However, happen it does, as witnessed by the potent mutator phenotypes of cells that lack the proofreading activity (Schaaper and Radman, 1989). It is probable that the mispairs most likely to escape proofreading are those that present the lowest kinetic and structural barrier to primer extension, such as G•T. These have to be eliminated by the last bastion of genomic integrity, postreplicative mismatch repair. In light of the above findings, it is no surprise that the G•T mispair is very efficiently bound by both prokaryotic (Jiricny et al., 1988) and eukaryotic (Hughes and Jiricny, 1992; Marsischky and Kolodner, 1999) mismatch binding factors. The structure sought by the mismatch recognition factors is most likely the wobble base pair, as this was the conformation seen to exit the duplex binding site of the polymerase (Johnson and Beese, 2004), but this pairing becomes grossly distorted once the G•T mispair is bound, as shown for the MutS mismatch binding protein of *E. coli* (Lamers et al., 2000).

Although we are still a long way from understanding all the molecular transactions involved in mismatch formation and repair, Johnson and Beese (2004) have brought us a big step closer to understanding how infidelity happens. As to how mismatch repair covers up the traces of erring polymerases, that's another story. Stay tuned….

Josef Jiricny
Institute of Molecular Cancer Research
University of Zürich
August Forel-Strasse 7
CH-8008 Zurich
Switzerland

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