Geology's latest gift to molecular biology?

Only a few hardy bacteria and archaea make their home in geothermal springs, but the enzymes of these thermophiles have for decades benefitted biological research. While the value of the bacterial DNA polymerase “Taq” in amplifying DNA (*via* PCR) is well known, DNA polymerases from hyperthermophilic archaea have been helping biochemists and structural biologists unravel a more subtle and complex problem: How do cells minimize the genetic consequences of DNA damage?

For all cells, damage to DNA is life-threatening yet unavoidable. The best response is to repair it before the DNA needs to be replicated, but cells also need a back-up system allowing DNA replication to cope with any damage that escapes repair. When DNA replication encounters an unrepaired lesion and stalls, one such system, called trans-lesion DNA synthesis (TLS), brings in specialized DNA polymerases that continues strand synthesis opposite the damage. Because it affects DNA replication, TLS contributes to processes ranging from genetic diversification to cancer chemotherapy.

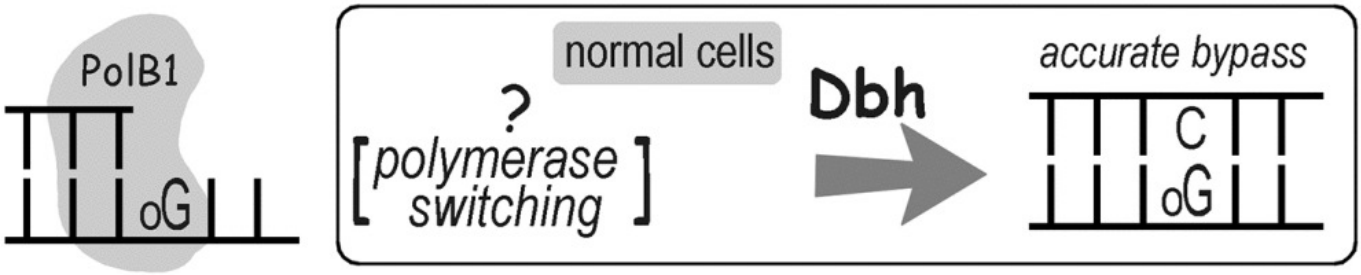
Sulfolobus species are archaea that colonize acidic hot springs; their TLS DNA polymerases crystallize well, which allows X-ray diffraction to create 3-D snapshots of TLS accurate at the atomic level. The results show that, unlike most DNA polymerases, the *Sulfolobus* TLS enzymes have large active sites that accommodate diverse DNA lesions; they also lack the “proofreading activity” that normally removes mis-aligned bases. Although logical for TLS, these two properties also compromise accuracy. The TLS polymerase from *Sulfolobus acidocaldarius*, for example (designated “Dbh”), makes mistakes on about half of the undamaged DNAs that it copies.

Can such an inaccurate DNA polymerase defend a cell’s genetic integrity? Developing genetic techniques for *S. acidocaldarius* has allowed this question to be addressed for the first time.

A DNA polymerase as erroneous as Dbh should create spontaneous mutations at a high rate in

living cells. However, removing this enzyme from *S. acidocaldarius* using gene-deletion techniques did not change the overall mutation rate, and actually increased a particular type of mutation in which G:C basepairs are replaced by T:A. Interestingly, these mutations commonly arise when guanine bases in DNA are oxidized and the resulting damaged form (oxoG) is replicated by "regular" DNA polymerases.

Does *Sulfolobus* use an inaccurate DNA polymerase to replicate oxoG accurately?



Sakofsky and Grogan recently addressed this question by placing oxoG into the chromosomes of normal *S. acidocaldarius* cells and those lacking Dbh, then identifying what DNA base was inserted opposite the damaged base. The results confirm that Dbh somehow competes successfully with three other DNA polymerases in *Sulfolobus* cells to insert C opposite oxoG; this prevents the original genetic information from being compromised by chemical damage. When Dbh is missing, the other DNA polymerases insert 'A' and other bases, creating mutations.

How do *S. acidocaldarius* cells recruit the Dbh polymerase specifically to oxoG, and how do they prevent it from replicating undamaged DNA (which is much more abundant in cells)?

This topic of future research is especially interesting because bacteria and eukaryotes use drastically different mechanisms to co-ordinate DNA polymerases at sites of damage, whereas archaea represent yet a "third form" of cellular life. Like bacteria, archaeal cells are small and simple, but their DNA replication proteins correspond to those of eukaryotes. Despite this relatedness, however, archaea do not have the ubiquitin-based signaling system that eukaryotic cells use to control TLS. So, it remains unclear whether archaea use the bacterial, the eukaryotic, or their own uniquely archaeal strategy to orchestrate TLS. It does seem clear, however, that answering this question will exploit *Sulfolobus* species not simply as a source of enzymes, but as a genetic resource with a uniquely geothermal origin.

Publication

Lesion-Induced Mutation in the Hyperthermophilic Archaeon Sulfolobus acidocaldarius and Its Avoidance by the Y-Family DNA Polymerase Dbh

Sakofsky CJ, Grogan DW
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