OVERVIEW

Cells endure the *continual generation of DNA damage*. Numerous lesions are generated through hydrolytic decay of DNA. Predominant such lesions are sites of purine base loss (**AP sites**), and sites of base deamination, particularly of cytosine (generating uracil in DNA) and adenine (to produce hypoxanthine). AP sites strongly disrupt DNA replication and, because they lack genetic information, are mutagenic when they are copied. The deaminated bases are mutagenic (U pairs with A rather than G; hypoxanthine codes ambiguously). The hydrolytic DNA lesions are generated at a rate of at least 10,000 per day in every mammalian cell, even under ideal conditions, and so constitute a strong threat to cell function and genetic stability. Moreover, the hydrolytic reactions are all accelerated in single-stranded DNA, as generated in normal cellular processes such as replication and transcription. Beyond the problem of hydrolytic damage, DNA reacts with metabolic by-products, most notably oxygen radicals. The estimates for the production of "oxidative DNA damage" are at least as high as those for hydrolytic lesions; other metabolites also produce damage in DNA. Processes such as inflammation generate still more oxidative DNA damage. The ~100 different oxidative lesions are also mutagenic, and they disrupt replication and transcription.

It is thus remarkable that, despite this nonstop genotoxic onslaught, cells normally exhibit genetic stability. The chief reason is that multiple DNA repair pathways and cell-cycle control systems cope with this damage to prevent mutations and chromosome aberrations. The key pathway for endogenous DNA damage is base excision DNA repair (**BER**). BER is initiated by the hydrolytic generation of AP sites, or by DNA glycosylases, which excise aberrant bases to generate AP sites. In mammalian cells, the next step of AP site incision is carried out almost entirely by Ape1 protein, which we isolated and whose gene we have cloned and characterized. Ape1 generates a normal 3'-OH nucleotide, enabling DNA repair synthesis. DNA polymerase β (**Polβ**) is generally responsible for this step, and a second active site excises the abasic residue left by Ape1 on the downstream 5' terminus. That allows DNA ligation, provided that no more than a single nucleotide has been inserted by the polymerase, producing the very efficient "short-patch" (more correctly "single-nucleotide") BER (**SP-BER**). An alternative "long-patch" pathway, **LP-BER**, involves more extended repair synthesis, estimated as 2-12 nucleotides, and perhaps involving the replication DNA polymerases δ and ε. The function of LP-BER remained obscure until we found that, during attempted SP-BER of the oxidative lesion 2-deoxyribonolactone, Polβ is covalently trapped while attacking the 5'-abasic residue. LP-BER avoids the protein-DNA crosslink problem and accounts for *all* of the 2-deoxyribonolactone repair in cell extracts: a naturally-occurring DNA lesion requires LP-BER. Nonetheless, crosslinks of Polβ and other repair proteins to DNA occurs in cells treated with certain oxidants, indicating that LP-BER in vivo is limiting.

We came to realize that actual SP-BER and LP-BER have never been followed in vivo. Consequently, new approaches are needed to establish how these pathways are actually deployed in intact cells. We recently published one approach using a mass-labeled probe but the procedure is inefficient, time-consuming, has a low yield, and includes many limitations. Here I propose a new approach will both streamline the preparation of probe substrates, expand the DNA lesions that can be interrogated, and allow for expanded analysis around the site of BER. Success in this effort will be a "game-changer" for a very fundamental problem in biology and human health, as well as in the understanding of evolutionary processes.