DNA replication can be likened to a car travelling down a long highway. Normally the road is paved smooth, but, as often occurs during travel, the road sometimes contains bumps and roadblocks. Such is the case for the replication machinery as it travels down DNA. While DNA damaged by natural and manmade agents normally is repaired by a multitude of repair pathways, some lesions inevitably escape repair and, as a result, are encountered by the DNA replication machinery. Many of the lesions cannot be bypassed by the replicative DNA polymerases and will lead to cell death if not overcome. Just how cells handle these road blocks has been a longstanding problem.

Upon encountering a lesion, the replicative polymerase may dissociate from DNA, leaving a gap in the newly synthesized strand. Such a gap could be filled in by a recombinational mechanism (1) or by a “copy choice” type of DNA synthesis (2). Because both of these mechanisms use information from the undamaged sister duplex to fill in the gap, they are relatively error-free. However, replication of damaged DNA also can occur by synthesis across the lesion from the template strand. In this case, a specialized replication complex inserts a random nucleotide across from the lesion and continues synthesis beyond the lesion. This process is usually mutagenic and is best exemplified by the *Escherichia coli* SOS system in which the complex of UmuC and UmuD proteins interacts with DNA polymerase III (PolIII) helicosome to promote damage bypass. Until recently, the prevailing notion for UmuC-UmuD action has been that it overrides the normally high fidelity of PolIII, enabling PolIII to insert a nucleotide across from the lesion and to replicate past the lesion (3).

UmuC belongs to a protein family that includes *E. coli* DinB, *Saccharomyces cerevisiae* Rev1 and Rad30, and human hRad30 proteins. In this issue of PNAS, Gerlach et al. (4) identify another member of this family in humans, which they refer to as DinB1. Recent studies of members of the UmuC protein family have heralded the emergence of a new paradigm of damage bypass in which, rather than modifying the activity of the replicative DNA polymerase, these proteins themselves are DNA polymerases specific for bypassing lesions in a mutagenic or error-free manner (Table 1). The first hint of this new paradigm came from studies in *S. cerevisiae*, where genes belonging to the *RAD6* epistasis group function in error-free and error-prone damage bypass. The *REV1*, *REV3*, and *REV7* genes of this group are essential for mutagenic bypass, and *RAD5* and *RAD30* function in error-free bypass of UV-damaged DNA. Rev1 possesses an unusual deoxyctydylidyl transferase activity that specifically inserts a dCMP residue opposite a template abasic site (5). Rev1 also can insert a cytosine opposite template guanine, adenine, or uracil, but it does so with a much reduced efficiency (10–20%). The insertion of cytosine opposite the abasic site produces a terminator that can be extended by DNA polymerase ξ, comprised of the Rev3 and Rev7 subunits (6). Thus, mutagenic bypass of abasic sites in yeast could occur by the coordinated action of a transferase and an error-prone DNA polymerase (Fig. 1).

Although the deoxyctydylidyl transferase activity of Rev1 is template specific, Rev1 is clearly distinct from classical DNA polymerases in that it inserts only a cytosine residue. The first member of this family of proteins shown to be a bona fide, classical DNA polymerase was *S. cerevisiae* Rad30-encoded Polη (7). Unlike Rev1, Polη synthesizes DNA by incorporating all four nucleotides in a template-specific manner. Deletion of *RAD30* results in moderate sensitivity to UV light, and deletion of both *RAD5* and *RAD30* causes a synergistic increase in UV sensitivity and in UV mutagenesis, implicating these genes in alternate error-free bypass pathways (8–10). By contrast to error-prone synthesis by the Rev proteins, Polη bypasses a thymine-thymine (T-T) dimer, a major UV photoprotein, in an error-free manner by inserting two A residues opposite the two Ts of the dimer (7) (Fig. 1). The sun-sensitive cancer-prone syndrome xeroderma pigmentosum (XP) can arise from a defect in nucleotide excision repair (NER) or from a defect in the replication of UV-damaged DNA. Cells from the variant form of XP (XP-V) have no defect in NER, but they are unable to replicate UV-damaged DNA (11–13). Additionally, XP-V cells are hypermutable with UV light, and they are less likely than normal cells to incorporate adenesines opposite thymine photoproducts (14, 15). These observations, and the ability of the yeast enzyme to faithfully replicate dimer-containing DNA, prompted the proposal that mutations in human Polη would cause XP-V (7).

The gene for the human RAD30 counterpart, hRAD30A, recently was identified by two different groups and shown to harbor nonsense or frameshift mutations in cell lines derived from XP-V patients (16, 17). Because the majority of such mutations in *hRAD30A* would produce severe truncations of the protein and result in the loss of function, *hRAD30A* is not essential for viability or for growth. Like its yeast counterpart, hRAD30A-encoded Polη bypasses a T-T dimer (17). In the absence of error-free replication by Polη, error-prone translesion synthesis by Polη would cause hypermutability and result in increased incidence of cancers that occurs in XP-V patients.

Another human RAD30 homolog, hRAD30B, recently has been identified (18). The gene is located on chromosome 18q21.1, a region that frequently is deleted in many cancers. It remains to be seen whether mutations in this gene contribute to any of these cancers. The *hRAD30B* transcripts are highly expressed in testes and to a lesser extent in heart and pancreas. The human homolog of *E. coli* DinB, hDINB1, is located on chromosome 5q13.1. *hDINB1* is expressed in a variety of tissues, and its expression is also highest in testes (4). The high level expression in testes may reflect a specific role of these genes in some aspect of spermatogenesis or spermiogenesis.

In *E. coli*, mutagenic bypass of abasic sites recently has been reconstituted by using purified UmuC, UmuD’, activated RecA, β-sliding clamp, γ-clamp loading complex, single-stranded DNA-binding protein gDINB1, and helicosome. This system also can be reconstituted in vitro using purified proteins, or it can be reconstituted with the purified enzyme. This system is highly dependent on the gDINB1 protein, which is necessary for the initiation of replication. When gDINB1 is replaced by the purified enzyme, replication is much reduced efficiency (10–20%).

*To whom reprint requests should be addressed: Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, TX 77555-1061. E-mail: lprakash@scms.utmb.edu.

See companion article on page 11922 in issue 21 of volume 96.
Modes of bypass replication in *E. coli* and *S. cerevisiae*. As DNA replication proceeds, the replicative DNA polymerases, PolIII in *E. coli* or PolI in *S. cerevisiae*, encounter lesions or stall sites (triangles) in DNA. In *E. coli*, the DNA polymerase activity of the UmuD’·C complex carries out limited DNA synthesis across and past the lesion in an error-prone manner. At replicative pause sites, such as at a misaligned template-primer junction, the DinB protein could carry out limited synthesis, resulting in −1 frameshifts. In *S. cerevisiae*, the Rad30A-encoded PolIγ performs replicative bypass of thymine dimers in an error-free manner by the insertion of two adenines across from the dimer. Alternatively, the Rev1 protein together with the Rev3/Rev7-encoded Polη carries out replicative bypass of UV lesions and other base damages in an error-prone manner. In humans, defects in PolIγ in XP-V patients result in the loss of this error-free component of UV damage bypass. Elevated mutagenesis arising from increased bypass by Polη would be the underlying cause of high cancer incidence in XP-V patients.
products that are 1 or 2 nt shorter than the ones generated by the Klenow fragment. DinB may promote replication through pause sites in repetitive DNA sequences via template misalignment (Fig. 1).

Phylogenetic comparisons have indicated the existence of four subfamilies within this family of DNA polymerases. These subfamilies have been categorized as UmuC-like, DinB-like, Rad30-like, and Rev1-like (4). Although UmuC-like proteins have been identified only in Gram-negative organisms, the Rad30 and Rev1 subfamilies have been found in both prokaryotic and eukaryotic organisms. The Rad30 and Rev1 subfamilies have been found only in eukaryotic organisms. Alignment of some of these proteins from diverse organisms as E. coli, S. cerevisiae, Schizosaccharomyces pombe, Caenorhabditis elegans, and humans (Fig. 2) reveals the presence of the several highly conserved motifs, designated I-V. These motifs are likely involved in DNA binding and nucleotidyl transferase activities. In fact, mutations of acidic residues in motifs I and III in several of these proteins have been shown to inactivate the DNA polymerase activity (10, 21, 23). These alignments also show the presence of subfamily-specific conserved motifs that lie outside motifs I-V. For instance, in the hRad30A family, there is a highly conserved C2H2 zinc finger motif in the C terminus, and although the C termini of DinB1 subfamily of eukaryotic proteins also contain a zinc-binding motif, it is distinct from that found in hRad30A protein family (Fig. 2). The Rev1 subfamily contains highly conserved N-terminal and C-terminal extensions that are not found in other subfamilies. The unique C-terminal or N-terminal extensions present in these protein subfamilies could function in specific protein–protein interactions.

Although phylogenetic analyses place the newly identified hRad30B protein in the Rad30-like subfamily (18), a comparison of sequences indicates that the C terminus of this protein is distinct from any other protein in the various subfamilies. In addition, hRad30B contains a divergent GFDEx sequence in motif III, as opposed to the highly conserved S(1/L/V)DE sequence found in other members of this family. Because hRad30B has been found only in humans and mice, the entire genomes of S. cerevisiae and C. elegans have been sequenced and no ortholog has been identified), hRad30B may represent a new subfamily of proteins distinct from the Rad30A, DinB, UmuC, and Rev1 subfamilies.

The presence of DNA polymerase activities in the above-mentioned proteins has revealed a novel mechanism of damage bypass, conserved among prokaryotes and eukaryotes. These studies now raise a number of other questions that need to be addressed. For example: How are these alternative DNA polymerases coupled to the replicative machinery? Do these enzymes function in isolation, or are they part of multisubunit protein complexes? What is the range of DNA lesions that yeast and human Polη can bypass? Is Polη specific for the bypass of a T-T dimer, or can it bypass other UV lesions such as a T-C, C-T, and C-C dimer, and is Polη an “A” rule polymerase that bypasses some lesions correctly and others incorrectly? And finally, what are the roles of human Rad30B and DinB1 proteins? Do they function in error-free or in mutagenic damage bypass, and are they specific for different types of DNA lesions? It is hoped that answers to these and related questions will be forthcoming soon.