DNA repair: **Polymerases for passing lesions**

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Replicative DNA polymerases generally cannot pass lesions in the template strand. Now there is accumulating evidence for the widespread existence of a separate class of DNA polymerases that can carry out translesion synthesis in both mutagenic and error-free ways.

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The main replicative DNA polymerases are processive enzymes that are overwhelmingly faithful to their template. The price paid for this fidelity is an inability to cope with abnormalities in the template DNA, particularly damaged or missing bases. As damage is an inescapable part of the chemistry of DNA in aqueous solution that is replete with active oxygen and other reactive species, cells have developed a variety of ways of dealing with the problem. They have control systems — 'checkpoints' — to inhibit DNA synthesis in the presence of damage, and they possess a multitude of enzymes for removing lesions before synthesis resumes. Even so, the checkpoints are not perfect and there is always a fraction of damage that is refractory to repair and is eventually encountered by a replication fork (Figure 1).

When a polymerase encounters such unrepaired damage, it may stall and synthesis may reinitiate downstream, with the resulting gap filled by recombination repair. Sometimes this does not or cannot happen, and there remains a need for synthesis across the lesion in the template strand. One way of achieving this is exemplified by the 'SOS' mutagenesis system in *Escherichia coli*, in which DNA damage induces production of the proteins RecA, UmuC and UmuD; UmuD is subsequently processed by RecA to UmuD′. These proteins can then all interact with DNA polymerase III holoenzyme, enabling it to synthesise past template lesions — though at the cost of introducing errors. The SOS system is responsible for most of the mutagenicity resulting from DNA damage in *E. coli*.

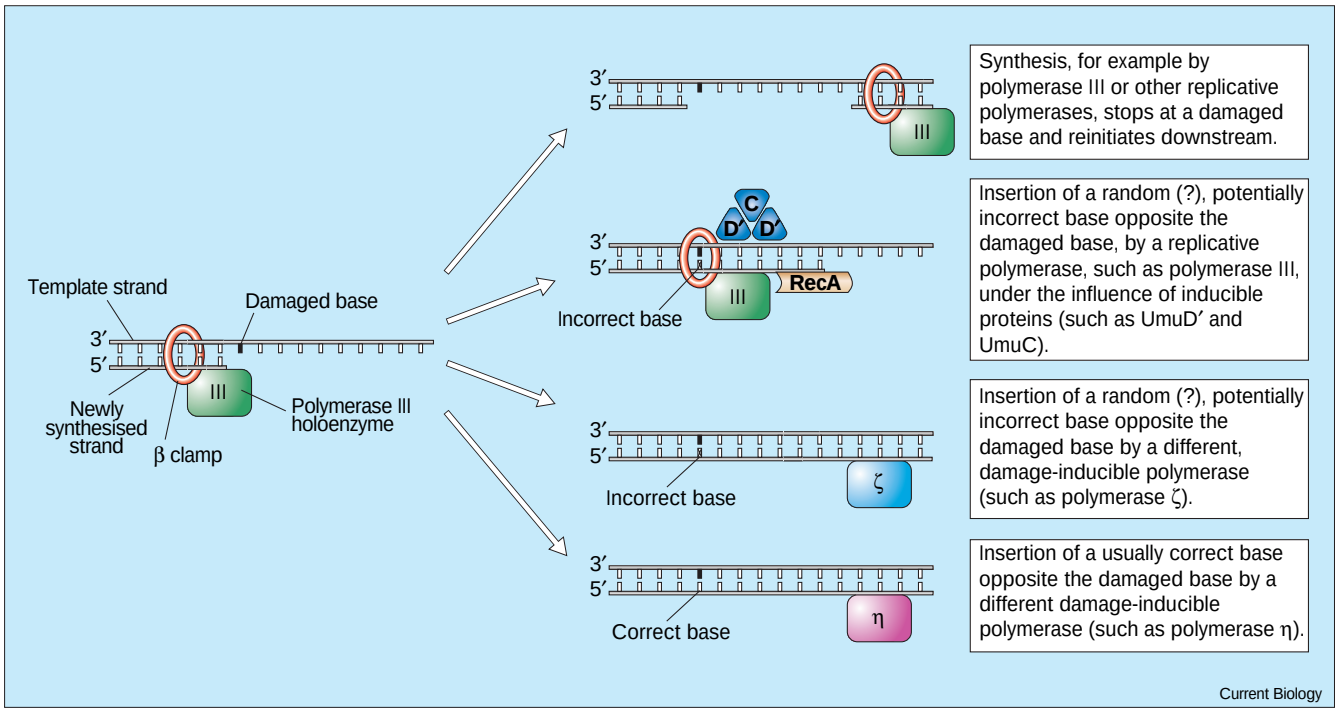
This process of translesion synthesis was recently successfully reconstructed *in vitro* (see [1]), following the purification of the UmuC protein. Of the two groups that achieved this, Tang *et al.* [2] found that their preparation of UmuC itself contained a low level of polymerase activity, sufficient to carry out some translesion synthesis in the reconstituted system lacking RNA polymerase III. Reuven *et al.* [3], on the other hand, observed an absolute requirement for RNA polymerase III. They, however, had purified their UmuC by tagging, in a way that might conceivably have inactivated any polymerase activity. So while their experiments showed that any UmuC polymerase activity was inessential for translesion synthesis, they did not rule out its presence in the normal protein.

The question of whether UmuC protein is itself an error-prone polymerase able to accept a damaged template is still unresolved, but such polymerases have been shown to be employed by other organisms. In the budding yeast *Saccharomyces cerevisiae*, the *scREV3* locus specifies the catalytic subunit of DNA polymerase ζ, a non-replicative polymerase which also contains the Rev7 protein and which, together with Rev1 and possibly other proteins, is thought to carry out translesion synthesis [4]. DNA polymerase ζ is responsible for almost all mutagenesis resulting from DNA damage in budding yeast [5]. The human homologue has been cloned and the protein, while twice the size of the yeast protein, has all the properties expected of an eukaryotic DNA polymerase [6]. Human cells expressing high levels of an *hsREV3* antisense RNA fragment grow normally, but show little or no mutagenesis after exposure to ultraviolet light, to which they are also somewhat more resistant. Polymerase ζ may therefore be a major source of mutations when human DNA suffers damage.

It would, however, be a mistake to think that all polymerases capable of translesion synthesis are error-prone. Evidence for error-free replication of DNA containing pyrimidine dimers in *E. coli* has existed for some time. In budding yeast, the damage-inducible *RAD30* gene is known to be involved in error-free post-replication repair [7]. The *RAD30* gene product has now been purified, and has been found to be a DNA polymerase — now called polymerase η, the seventh eukaryotic polymerase to be described — capable of inserting correct bases opposite a *cis-syn* cyclobutane thymine dimer, a lesion that blocks normal replicative polymerases [8]. Little is known at present about the range of DNA lesions that polymerase η can cope with, or how it manages to carry out translesion synthesis without introducing errors.

In the present context, the fact that *RAD30* was originally identified as a homologue of the *E. coli* gene *umuC* [7] — as is *REV1* — serves to refocus attention on the possible polymerase activity of the *E. coli* UmuC protein, as well as that of the protein encoded by another *E. coli* homologue of *umu C*, *dinB*. The *dinB* gene is known to be damage-inducible, and its protein product is known to have a role

Figure 1



Four ways of dealing with a damaged DNA base at the replication fork. For simplicity, the figure shows only the template strand with the damaged base. Homology exists between *E. coli* UmuD protein, yeast polymerase η and yeast and human polymerase ζ, suggesting that there is considerable mechanistic conservation between bacteria and humans.

in untargeted mutagenesis of bacteriophage lambda. DinB also appears to be widely conserved in nature.

Wagner *et al.* [9] have now demonstrated that *dinB* encodes a new *E. coli* DNA polymerase — named polymerase IV — and that this activity is necessary for the *in vivo* mutator effect observed when *dinB* is overexpressed in the cell. They have also shown that, *in vitro*, the DinB polymerase is capable of efficiently elongating a misligned primer–template substrate — which represents a frameshift mutation intermediate — a property that is in full agreement with the mutator spectrum observed *in vivo* when *dinB* is overexpressed and during the untargeted mutagenesis of bacteriophage lambda.

It has been known for some 25 years that cells from patients with the variant form of the sun-sensitive cancer-prone disorder xeroderma pigmentosum (XPV) have a defect in the replication of UV-irradiated DNA. The mutation frequency is elevated and the spectrum of mutations is different from that observed in wild-type cells. There have been several studies suggesting a defect in the replication of damaged double-stranded DNA, but a recent study has unequivocally shown that extracts from XPV cells are defective in the ability to carry out translesion synthesis past a single *N*-2-acetylaminofluorene (AAF) adduct at a defined position in single-stranded plasmid DNA [9].

The primary defect in the XPV extracts appears to be the inability to incorporate or stably maintain a nucleotide opposite the AAF adduct, a defect which reflects the *in vivo* situation. Could this be due to a defective or absent DNA polymerase of the UmuC family? Polymerase ζ — the *hREV3* gene product — is error-prone, and its absence would be expected to result in a lower, rather than higher, level of induced mutagenicity. Moreover, the human homologue of *scREV3* has been excluded as the gene defective in XPV (cited in [10]). It is not unreasonable to suppose that human polymerase ζ might be responsible for the error-prone translesion synthesis that appears to remain in XPV cells. The translesion synthesis that is defective in XPV cells seems likely therefore to be of the error-free variety, and a putative human homologue of the error-free *RAD30* has been proposed as a candidate gene [8]. Now that translesion synthesis can be studied with defined lesions in single-stranded DNA, we may anticipate rapid resolution of these speculations.

The more one learns about genes involved in DNA repair, the more one appreciates that they usually have multiple roles. Could one of these error-prone polymerases have an

additional role in immunoglobulin gene processing? In mice and humans, the somatic mutations that occur in processed immunoglobulin genes appear to arise *de novo*, and polymerase ζ has been suggested as a potential effector of this process [11]. The immune response in mice with deleted polymerase ζ subunits or associated proteins is in hand and should be revealing.

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