Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 24

DNA base excision repair (BER)

From nucleotide excision repair (NER), which was the topic of the previous chapter, we now move on to a related DNA repair process, namely base excision repair (BER) (Figure 24.1).

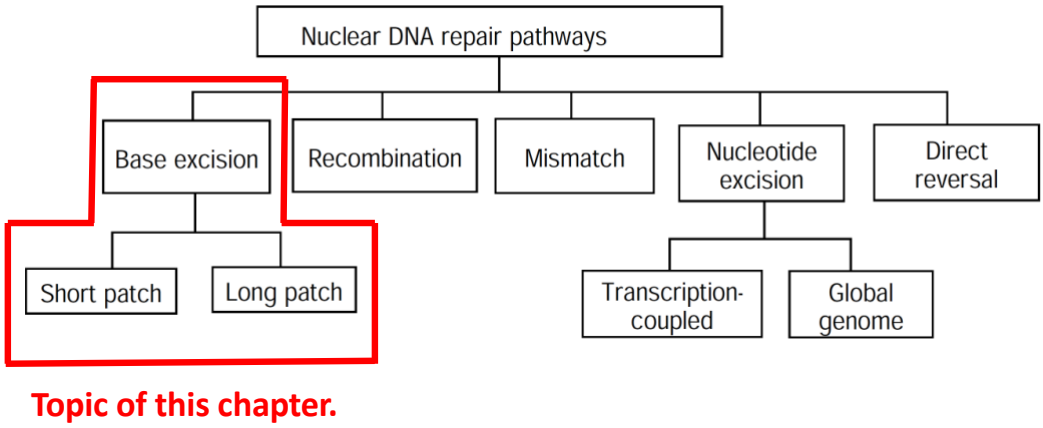

Figure 24.1. The topic of this chapter, base excision repair (BER), in relation to the other DNA repair pathways in the cell nucleus. *From (Kohn and Bohr, 2001)*.

The uracil-DNA glycosylase story

In 1974, Thomas Lindahl (Figure 23.2 in Chapter 23) reported his investigations of why DNA slowly loses its biological activity when it is left in a warm solution for a long time (Lindahl, 1974, 1976). DNA had been reported to suffer several types of chemical changes spontaneously at a rate that increased with increasing temperature. Lindahl focused on one of those chemical reactions that he thought likely to be biologically significant even in unstressed organisms. He focused on cytosine's amino group that very slowly comes off and is replaced by a hydroxyl group. This is a typical hydrolysis reaction in water solutions. The biological problem was that the slow reaction changed the cytosine to uracil, thereby changing the base-pairing: cytosine pairs with guanine while uracil pairs with adenine. Lindahl reasoned that evolution must have found a way to overcome that problem. Therefore, he searched for an enzyme might be able to remove the offending uracil whenever it reared its ugly head in DNA.

What he discovered was the first of a large group of enzymes, each of which can pluck off a particular abnormal base from DNA. The enzyme he discovered specifically removed uracil by means of a "glycosylase" reaction, which breaks the bond between one of uracil's nitrogen atoms and deoxyribose. The uracil-DNA glycosylase, as it came to be known, only removed uracil from DNA, but did not remove uracil from RNA, where it belongs. Nor did it remove thymine (which is like uracil, but with an extra methyl group) from DNA. Thus, the DNA-uracil glycosylase enzyme was carefully designed to act only where it is needed. Moreover, DNA has evolved to use thymine instead of uracil to pair with adenine in DNA, because uracil would be removed by the glycosylase. Evolution is a remarkable designer!

After an offending uracil is removed, it leaves behind a deoxyribose lacking any base. This is known as a base-free site in DNA. It is like a nucleotide with its head chopped off (left side of Figure 24.2). The base-free site that is left behind still needs to be repaired -- which is accomplished by previously discovered enzymes, as I will explain later in this chapter.

The early work on DNA glycosylases was done in bacteria. But Lindahl later remarked that the major DNA repair pathways are surprisingly similar between *E. coli* bacteria and mammals and that cells of higher organisms have not evolved any novel DNA repair pathways; moreover, that DNA repair mechanisms probably evolved very early, because they appear to be present in all living organisms (Lindahl, 1982). He noted that the most important of the DNA repair pathways, in *E coli* as well as in human cells, repair damaged bases, which are among the most frequent type of DNA lesion. The first step in this pathway would be to locate the abnormal base and then to cut it out of the DNA, leaving behind a base-free site that must then be repaired. That entire process has come to be known as base-excision repair (BER) to distinguish it from nucleotide-excision repair (NER), which was the subject of the preceding chapter. Errol Friedberg too had a major role in the discovery of BER and gives an interesting personal account of its history (Friedberg, 2016).

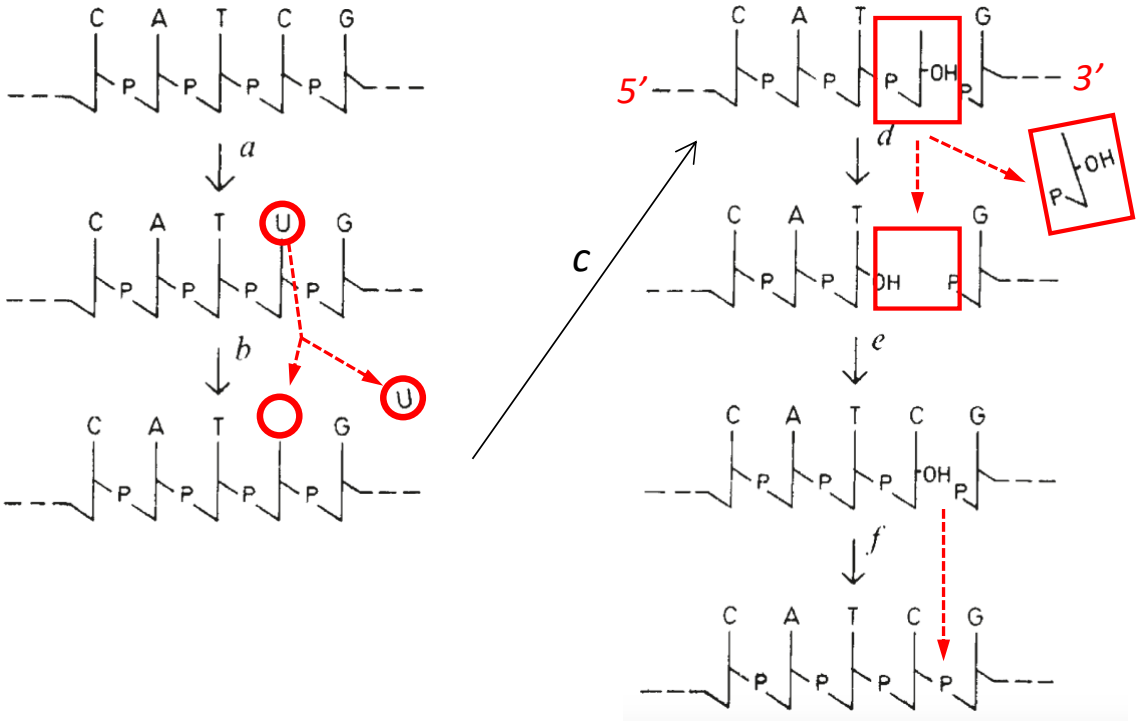

Figure 24.2. DNA base-excision repair (BER) as depicted by Thomas Lindahl in 1976 (Lindahl, 1976), with additions shown in red. The left side shows the removal of an offending uracil (U), leaving a base-free site. The right side then shows how the base-free site is repaired. In reaction *a*, hydrolysis of a cytosine's amino group changes the cytosine to uracil (U), which does not belong in DNA. In reaction b, DNA-uracil glycosylase cuts off the U and leaves behind a base-free site (empty red circle). In reaction c, an AP-lyase cuts the bond on the 3' side of the base-free unit (enclosed in a red box). In reaction *d*, an AP-endonuclease cuts the bond on the 5' side of the base-free unit, leaving a gap in the DNA strand (shown by the empty red box). In reaction *e*, a DNA-repair polymerase adds the correct nucleotide (C) to the 3' end of the DNA strand. In reaction *f*, a DNA ligase finally seals the C in place, thereby completing the repair. (The diagram omits the complementary DNA strand.)

Discovery of other DNA glycosylases.

Aside from uracil-DNA glycosylases, many enzymes were discovered that cut off other abnormal bases from DNA. By 1982, 15 DNA glycosylases had been discovered, each designed to remove a particular abnormal base from DNA, analogous to the removal of uracil shown in Figure 24.3 (Lindahl, 1982). The job of these enzymes is to cut the bond between the abnormal base and the deoxyribose sugar in DNA; the enzymes simply catalyze a hydrolytic cleavage of the bond and do not require energy or any cofactor. (Hydrolytic cleavage is a reaction that induces a water molecule to split a bond; an OH

becomes bound to one end of the split bond and an H to the other end.) Each glycosylase was found to act only on the abnormal base that evolution designed it to remove. ("glyc" refers to a sugar, in this case deoxyribose; thus, *glycosylase* means enzyme that cleaves a bond to a sugar.)

One of the early reports that BER can remove and repair chemically modified bases from DNA came from Jacques Laval of the *Institut Gustav-Roussy* in Villejuif, France, in 1977 (Laval, 1977). He found that BER can remove and repair 3-methyl-adenine (3-meA) from DNA that had been treated with an alkylating agent (methyl-methanesulfonate).

Here I cannot resist relating an anecdote about Jacques Laval. Sometime in the 1970s, my wife and I met Jacques at a DNA repair conference in Lyon, France. Jacques was as always ebullient, friendly and a great pleasure to be with and talk about DNA damage chemistry. At a meeting session one morning, he came over to chat and asked where we had dinner last night. With a tiny bit of reluctance, I said we had found this fine Chinese restaurant… He said, "What! Here you are in the culinary capital of the world with so many 5-star French restaurants, and you go to a Chinese?" Well, a day or two later, he came over and asked: "Where did you say that Chinese restaurant was located?" It seems that, when one is constantly immersed in fine things of a particular kind, one could eventually tire of them. But I think that none of us tired of the emergent story of DNA repair.

As of 2009, eleven of the DNA glycosylases had been isolated (Robertson et al., 2009). Figure 24.3 shows the chemical structures of several abnormal bases that could erroneously be incorporated into DNA by a DNA polymerase and then removed by one or another of the glycosylases. Since specific enzymes have evolved to remove these abnormal structures from DNA, one may suspect that organisms have frequently formed or encountered such structures.

After the uracil-DNA glycosylase, the next to be discovered and extensively studied was the glycosylase that removes an oxidation product of guanine (8-oxoG, also known as oxo^{8}G, Figure 24.3) (Klungland and Bjelland, 2007). 8-oxoG is an unavoidable by-product of normal oxidative metabolism in mitochondria. DNA polymerase can erroneously and easily incorporate 8-oxoG into DNA. But instead of pairing with C of the template strand as it should, the oxo^{8}G sometimes wiggles around in a manner that its alternative hydrogen bonding capability allows it to mis-pair with A or G (Figure 24.4). Such mis-pairings are a major cause of mutation.

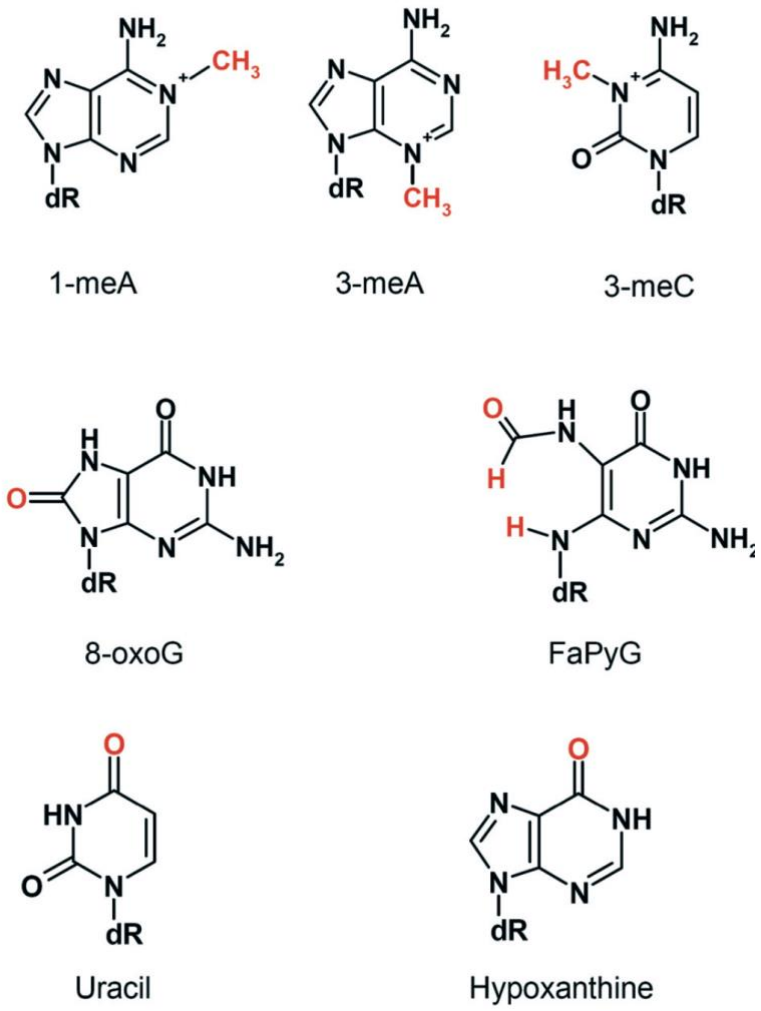

Figure 24.3. Some of the chemically altered bases that BER is capable of removing from damaged DNA (Robertson et al., 2009).

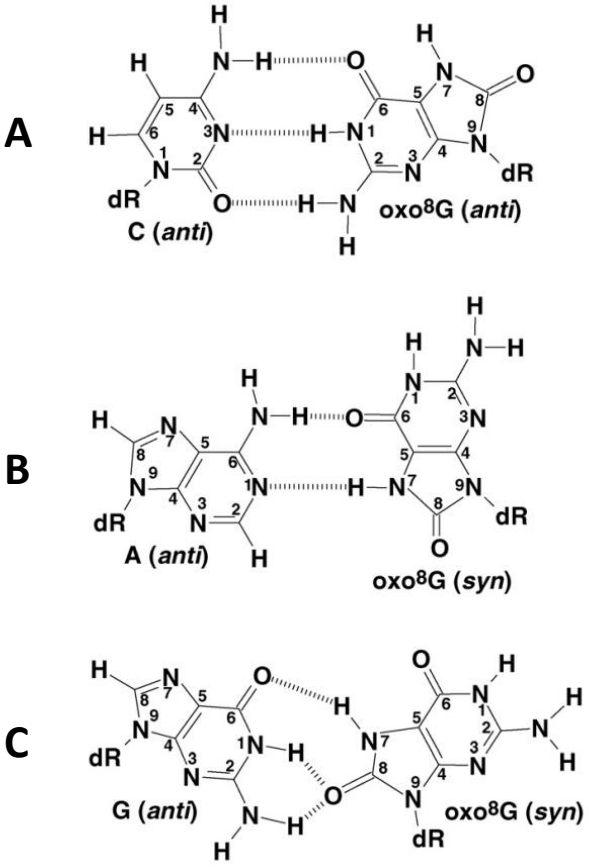

Figure 24.4. Oxidation processes of cell metabolism causes an oxygen atom addition to position 8 of guanine, which then allows the resulting 8-oxo-guanine to mis-pair with adenine or guanine. ***A***, pairing of 8-oxoG with C (like the normal G:C pair). ***B***, mis-pairing of 8-oxoG with A. ***C***, mis-pairing of 8-oxoG with G. (*From (Klungland and Bjelland, 2007)*).

Repair of base-free sites

The APE1 story

After base excision repair (BER) removes a bad base, a base-free site remains that has to be repaired. The first step in that repair is carried out by an enzyme that is so remarkable and so important that it became one of the most intensively studied of all enzymes. It came to be called APE1 (AP-endonuclease 1), although it had other confusing names as well. The enzyme was found to cleave the DNA strand in step *c* of Lindahl's 1976 diagram (Figure 24.2); it also contributed to step *d*.

Investigators soon realized that base-free sites actually were produced quite frequently. They were produced, not only during DNA repair, but also appeared spontaneously due to a slight instability of the bond that binds a purine or pyrimidine bases to the DNA. Lindahl estimated that a cell's DNA genome spontaneously loses about 10,000 bases per day. Since base-free sites occur so frequently, investigators searched for enzymes that might be implicated in their repair. In 1970, W. G. Verly and his coworkers at the University of Montreal, Canada, found an enzyme activity that cleaved DNA at or near a base-free site, and by 1973, they had purified the enzyme from both bacteria and mammalian cells (Verly et al., 1973). That is how APE1 was discovered.

However, it soon emerged that APE1 is more clever than just to cleave a base-free site: it also detects and removes mismatched nucleotides if any such mismatches exist at the cleavage site. Thus, it is not only and endonuclease (which cleaves an intact DNA strand) but also an exonuclease that chews away mismatched nucleotides from the end of a cleaved strand. Moreover, APE1 turned out to have even more functions than that: it was found to be at the nexus and central regulator of DNA repair in all its complexity. Furthermore, APE1 was found to be overexpressed in cells of a variety of cancers, which made it potential chemotherapy target (Fishel and Kelley, 2007).

How base-excision repair (BER) works.

A diagram of BER, as it was understood in 2009 (Robertson et al., 2009), is shown in Figure 24.5. It gives a more complete picture than was available in the 1976 diagram (Figure 24.2).

In the first step, a glycosylase removes the damaged base, leaving behind a base-free site. APE1 then cleaves the DNA strand on one side of the site, creating a 3'-OH end that is suitable for the DNA repair polymerase, POLB, to hook onto. POLB (DNA polymerase beta) specializes in DNA repair. It extends the broken DNA chain from the 3'-OH end of the break, adding one or more nucleotides.

Now, POLB is faced with a choice: whether to add only one or whether to continue adding several nucleotides. As of 2009, it was still unknown how the choice was made. In either case, however, the added nucleotides were chosen to match the complementary DNA stand, which is almost always what the major DNA polymerases do. The diagram suggests that the nucleotide addition continues when POLB hands off its work to POLD (Figure 24.5). The two branches of the BER mechanism each required a different set of enzymes and proteins; hence, the distinct mechanisms were awarded different names: *short-patch* versus *long-patch* repair.

The *short-patch* mechanism was obviously the simpler process. It involved relatively few enzymes and proteins. In addition to the enzymes already mentioned, a protein called XRCC1, which lacked enzyme activity, came into play. It was thought to function as a kind of scaffold that held together the needed enzymes, presumably helping to make the process

fast and efficient – which made sense, in view of the frequent occurrence of base-free sites reported by Lindahl.

But why was the more complicated *long-patch* process needed? In 2009 that was still a mystery (Robertson et al., 2009). One might speculate, however. A simple idea would be that POLB was occasionally too exuberant and continued adding nucleotides when it should have stopped after adding just one. Maybe that happens when the DNA strand it is facing happens to breathe. Breathe? Yes, the DNA double-helix would be expected occasionally and very transiently to come apart in regions where it is not locked in place by histones or other nuclear proteins. After having displaced and copied one more nucleotide than it should have, momentum might carry the polymerase forward until it runs out of steam and stops. Then a large collection of other proteins come in to fix this awkward situation where a displaced DNA single-strand is hanging out like a flap.

I will mention but a few of the many proteins that were implicated in the long-patch repair process diagrammed in Figure 24.5å (Robertson et al., 2009). The ring protein complex, PCNA, clamps like a donut around replicating DNA, carrying a DNA polymerase as it slides along, synthesizing a replicated double helix. RFC is a clamp loader that assembles the PCNA clamp around the DNA. The way this happens is of course complicated, but by 2012 it had all been worked out (Kelch et al., 2012). RPA binds and stabilizes the DNA single-strand segment that is displaced by the long-patch repair process, and FEN1 is a special nuclease that cuts off that flapped segment. Finally, LIG1 is a DNA ligase that seals the end of the newly synthesized DNA segment to form an intact strand.

The whole process, including the replication of the two DNA strands, was found to be similar in life forms from bacteria to humans; it is an astonishing accomplishment of evolution. Although long-patch repair has to replicate a segment of only one of the DNA strands, it seems to need much of the same machinery as replication that duplicates the cell's DNA during the cell cycle. Long-patch repair, however, needs additional factors to take care of the displaced DNA single-strand segment, such as BLM and WRN, which are implicated in homologous recombination, and the MSH proteins that are implicated in DNA mismatch repair (Robertson et al., 2009).

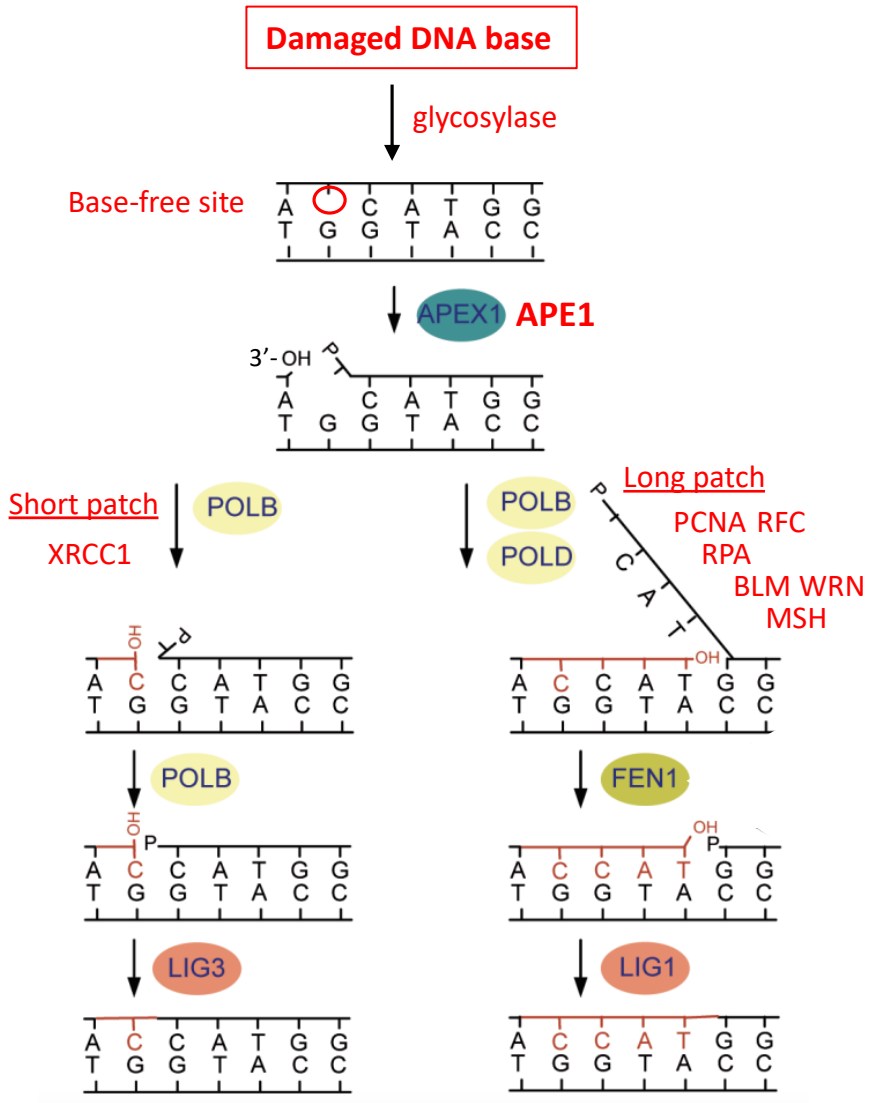

Figure 24.5. Steps in the repair of base-free sites after removal of an altered or damaged base from DNA where there is a damaged base. *Modified from (Robertson et al., 2009)*.

BER helps repair a topoisomerase-I (TOP1) blockage at a DNA lesion.

Base-excision repair (BER) can help repair a variety of other DNA damage problems. A recent example comes from a collaboration that included several members of Yves Pommier's laboratory at NCI (Saha et al., 2020). The problem arises when TOP1 in its strand opening and closing reaction (see Chapter 11) encounter a DNA lesion. The problem and its solution are depicted in Figure 24.6 and explained in its legend.

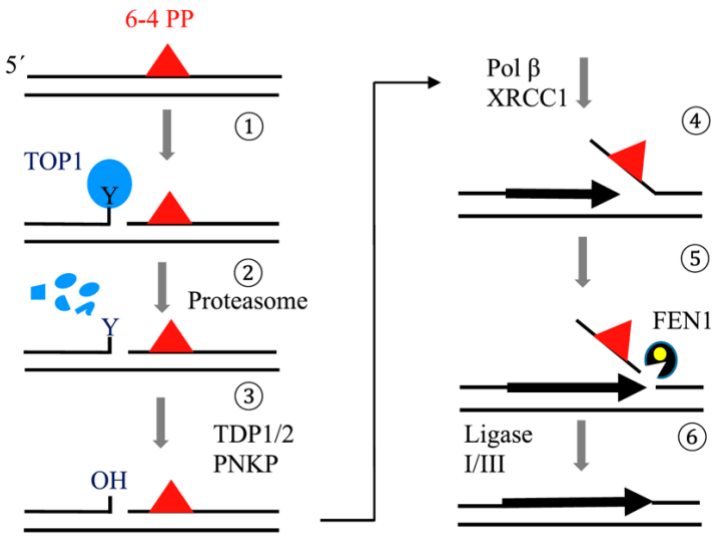

Figure 24.6. A DNA repair problem arising when TOP1 encounters a DNA lesion, in this case 6-4 pyrimidine dimer (Saha et al., 2020). **In step 1**, TOP1 has cleaved the DNA strand adjacent to a 6-4 PP and has bound to one end of the break by way of a tyrosine (Y) (see Chapter 11). The TOP1 is now trapped and cannot proceed. **In step 2**, a proteasome cuts away most of the trapped TOP1 protein. **Step 3** removes the remaining peptide, including the Y, leaving a 3'OH. **In steps 4-6**, long-patch BER comes into play and completes the repair, as in Figure 24.5.

The brief life of "DNA insertase".

Because glycosylase enzymes capable of removing improper bases from DNA, it seemed plausible that there might be an enzyme able to carry out the reverse reaction. The enzyme would directly insert the proper base into a base-free site. The existence of such a "DNA insertase" was reported in 1979. The following year, however, Errol Friedberg, working in Tom Lindahl's laboratory was unable to confirm the existence of such an enzyme. Subsequent reports suggested that the apparent "insertase" activity resulted from a combination of an enzyme that cleaved the base-free site and a polymerase that inserted the proper base – essentially like the short-patch repair on the left side of Figure 24.5. Doubts about insertase had already been raised, because the direct insertion of a base would require energy, whereas the reported reaction did not seem to need any. No further reports of DNA insertase activity appeared after the early 1980's, and so the albeit attractive insertase idea was quietly laid to rest (Friedberg, 2016).

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