Chapter 23. The DNA nucleotide excision repair story 220725ay3

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

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CHAPTER 23

The DNA Nucleotide Excision Repair Story: cutting out the damage.

Evolution has come up with an amazing set of tools to repair the great variety of damage that DNA can accrue. The repair tools can sustain life despite damage from radiation, environmental chemicals, chemotherapy, metabolic errors, reactive oxygen species from aerobic metabolism, cosmic rays, etc. The major sites of chemical damage to DNA, as understood in 1993, are shown in Figure 23.1. In view of these hazards, Tom Lindahl surmised that specific mechanisms must exist to repair those various kinds of damage to DNA (Lindahl, 1982). Lindahl shared the 2016 Nobel Prize in Chemistry with Aziz Sancar and Paul Modrich for their discoveries of DNA repair mechanisms (Figure 23.2) (Cleaver, 2016; Orren, 2016; Van Houten, 2016; Zagorski, 2005). The various DNA repair mechanisms are outlined in Figure 23.3, which also indicates in the legend the chapters that discuss each of them.

The current chapter delves into the tools that repair the greatest variety of chemical damage to DNA: nucleotide excision repair (NER), which cuts out a damaged piece of DNA strand and replaces it with a good piece. There are two types of NER: global-NER and transcription coupled NER (TCNER). Global-NER repairs DNA damage that may be present anywhere in the genome, whereas TCNER repairs DNA damage at sites where RNA polymerase is transcribing the genome. Genetic defects in NER cause xeroderma pigmentosum whereas defects in TCNER are associated with Cockayne's syndrome (Chapter 22).

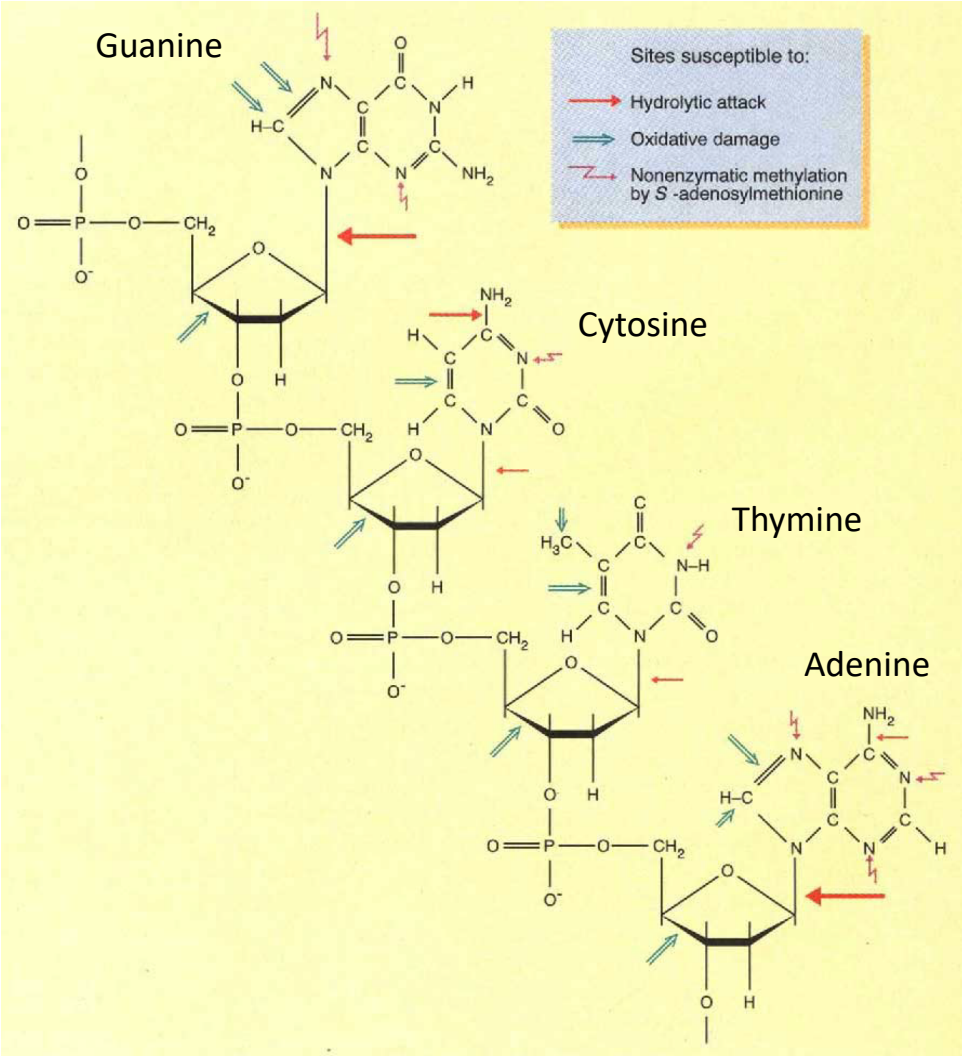

Figure 23.1. Sites where DNA is vulnerable to chemical damage, as summarized by Tomas Lindahl is 1993. The large red arrows point to the bonds in guanine or adenine (the purines) that are vulnerable to cleavage, thereby dislodging the guanine or adenine base from the DNA. The smaller red arrow pointing to the bond at cytosine's amino (HN2) group indicated where hydrolysis can replace the amino group by an oxygen atom, thereby converting cytosine to uracil. The open blue arrows show where oxidative damage can occur; those that point to the double-bond of thymine or cytosine show where UV light pairs them together as thymine dimers and thymine-cytosine dimers, collectively known as pyrimidine dimers (Lindahl, 1993).

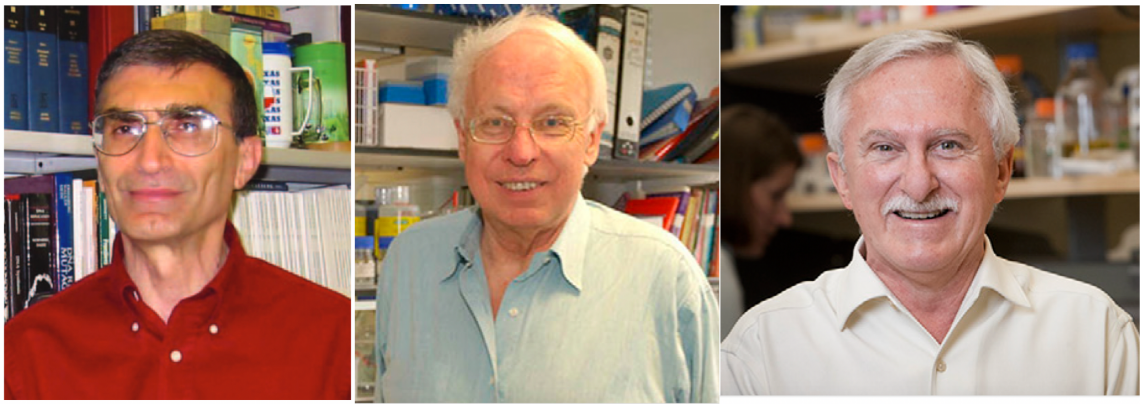

Figure 23.2. The three scientists who shared the 2016 Nobel Prize in Chemistry for their discovery of mechanisms by which human cells repair DNA damage. **Aziz Sancar**, born on 8 September 1946 in the Mardin Province of southeastern Turkey, received an MD degree at Istanbul University in 1969 and a PhD in molecular biology at the University of Texas at Dallas in 1977. He was elected to the National Academy of Sciences in 2005 as the first Turkish-American member. He discovered several DNA repair enzymes, elucidated how they work, and made notable discoveries also in other areas of cell biology. **Thomas Robert Lindahl**, born on 28 January 1938 in Stockholm, Sweden, received a PhD in 1967 and an MD in 1970, both from the Karolinska Institute in Stockholm. He isolated a mammalian DNA ligase that operates in NER and discovered a the totally unanticipated DNA glycosylases as mediators of DNA base excision repair. **Paul Lawrence Modrich**, born on 13 June 1946 in Raton, New Mexico, received a Ph.D. degree from Stanford University in 1973 He has been working at Duke University, Durham, North Carolina since 1976 and is also affiliated with Howard Hughes Medical Institute, Chevy Chase, Maryland. He discovered DNA mismatch repair and was elected to the National Academy of Medicine and the National Academy of Sciences.

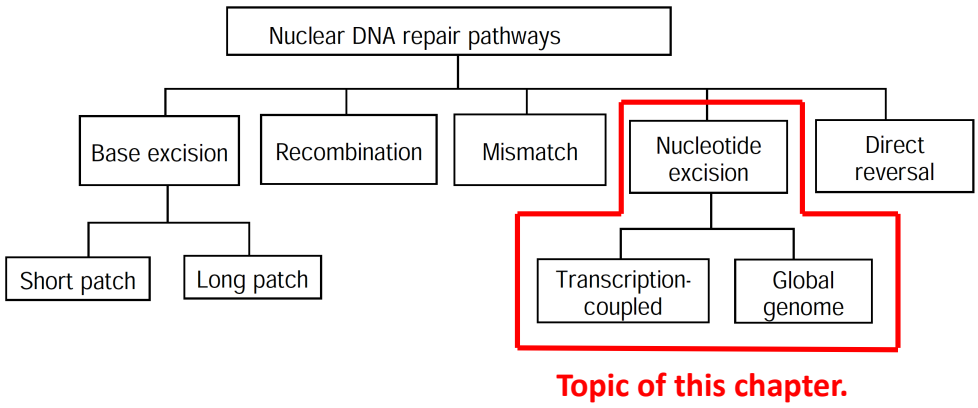

Figure 23.3. An overview of the types of DNA repair mechanisms operating in the cell nucleus. The topic of this chapter is outlined in red. *From (Kohn and Bohr, 2001)*. Base excision repair will be the topic of Chapter 24; recombinational repair, Chapter 26; mismatch repair, Chapter 25; nucleotide excision repair (NER), this chapter; direct reversal was the topic of Chapter 2.

Nucleotide excision repair (NER): cutting out and mending damaged DNA.

An evolutionary solution to the wide variety DNA damage might seem easy: simply cut out the damaged section of the DNA strand and patch the resulting gap with new DNA. Easily said -- which is not to imply that the "blind watchmaker" said or thought anything at all -- but it must have taken a great deal of more-or-less random evolution to design (or, rather, to select) the chemical and enzymatic processes to carry it out.

Nucleotide excision repair (NER) was perfected eons ago in the simplest microorganisms, possibly even before or during the time when the accumulation of oxygen from photosynthesis became the most toxic environmental mutagen in the history of our planet. Some DNA repair genes and mechanisms in animals might have arisen by gene transfers or incorporation of whole organisms (as in the case of mitochondria) from the microbes that originally evolved the repair machinery.

The major NER genes were discovered in the course of investigations of the various complementation groups of xeroderma pigmentosum (XP), as I explained in Chapter 22. They became known as XPA through XPG, plus a variant called XPV. These genes were listed in Table 22.2 in the previous chapter, but is reproduced here for convenience:

Table 23.1. Xeroderma pigmentosum genes (complementation groups)

Gene	Synonyms	Chromosome
XPA	XP1	9q22.33
XPB	ERCC3 (excision repair 3, helicase subunit)	2q14.3
XPC	RAD4	3p15.1
XPD	ERCC2 (excision repair 2, helicase subunit)	19q13.32
XPE	DDB2 (damage-specific DNA binding 2)	11p11.2
XPF	FANCQ, RAD1, ERCC4 (excision repair 4, endonuclease)	16p13.12
XPG	ERCC5 (excision repair 5, endonuclease)	13q33.1
XPV	XP variant, DNA polymerase eta	6q21.1

(Information from the human gene nomenclature committee (HGNC) website.)

Repair by cutting out damaged sections of DNA: a new idea.

In his review of the "very early history of nucleotide excision repair," Errol Friedberg says that this new idea "was much in the air in the 1950s and early 1960s and was circulating freely in the informal grapevine of seminars and meetings" (Friedberg, 2011). Those airy ideas also circulated freely among us in 1960 in Paul Doty's laboratory at Harvard, as well as among our colleagues at nearby Brandeis University. Friedberg cites a "prophetic notion" by Evelyn Witkin in the early 1960s, based on observations of the induction of certain mutations by UV light in bacteria, that "some type of enzyme-catalyzed dark repair" was involved.

Friedberg goes on to cite a report in 1963 by Robert Haynes that, if yeast irradiated with x-rays or UV were left for a few hours of delay before being allowed to grow on an agar surface, they were able to form a greatly increased number of colonies; it seemed that the yeast were repairing the damage during the delay period. A related, at first puzzling, observation was that, if the same dose of x-rays was delivered to bacteria, but spread out in time, the bacteria survived much better; it was soon grasped that repair was taking place during the protracted x-ray treatment.

But the first experimental evidence for DNA excision repair was obtained by Richard Setlow. Friedberg cites Haynes recalling that in the Fall of 1963, Setlow visited him at the University of Chicago and triumphantly asked "Do you know how E. coli repair [UV-damaged] DNA?" When Haynes replied "No, how?", Setlow said "They cut out the [thymine] dimers and throw them away!" A lucky break, Setlow said, was his early introduction to thymine dimers, and he recalled that "One of the people on the [Oak Ridge] staff approached me one day and asked what I thought about the experiments carried out by 'those crazy Dutchmen'?" referring to the experiments of Beukers and Berends showing the production of dimers by UV-irradiating frozen solutions of thymine (see Figure 21.15 and associated text in Chapter 21). On the basis of experiments showing that, after being irradiated with UV, bacteria gradually released small pieces of DNA containing the thymine dimers, "throwing them away," Setlow surmised correctly that there must be two cutting events -- it turned out later that the cuts are produced by two of the XP enzymes: XPF and XPG: a left-side cutter and a right-side cutter, respectively (Friedberg, 2011).

I found a picture of Richard Setlow that shows his bright interest in all there is in the nature world (Figure 23.4). I don't know what was swimming in the small fish tank he was holding: maybe it was a species whose DNA repair peculiarities he was studying. When I visited him at his home on Long Island in the 1970s or 1980s I felt his warm friendly informal manner – how he prepared an excellent dinner ad hoc without fuss and invited my collaboration in that enterprise; the relaxed intense way he wanted to talk about all kinds of things; it was hard to keep up with his thinking. It was only on reading his obituary that I learned that he stemmed from The Bronx, New York, where I too grew up a decade later. It is remarkable how many well-known people I met later in life or read about were from The Bronx of that era.

A next step in the saga was made by a former graduate student of Setlow's: Philip Hanawalt, who was by then on the faculty at Stanford University. His experiments are a bit complicated, and we don't need the details here; they are summarized by Friedberg (Friedberg, 2011). The story was essentially this: some time in 1963, Hanawalt called Setlow to tell him about some puzzling findings he had made. Setlow responded in a letter, dated August 23, 1963, telling him that his (Hanawalt's) findings were in accord with new results in his own laboratory and showed that UV-resistant E. coli strains had enzymes to cut the damage out of their DNA and that a UV-sensitive mutant E. coli could not do so, perhaps because the hypothetical "repair enzymes" whose job it was to cut out the damaged pieces of DNA were defective in those bacteria. Hanawalt said it was the first time he heard the word "repair" used in this context (Friedberg, 2011). The following year, Hanawalt and his student David

Pettijohn, published a paper with the title: "Evidence for the repair-replication of ultraviolet-damaged DNA in bacteria" (Pettijohn and Hanawalt, 1964).

A third laboratory that was simultaneously homing in on DNA repair in bacteria was that of Paul Howard-Flanders, a noted radiobiologist at Yale. The interactions between the two laboratories were complex and sometimes competitive, as Friedberg described them, (Friedberg, 2011). Finally, we should note that almost all of those advances were facilitated by making use of the radiation-sensitive mutant E. coli B_{s1} strain that had been isolated by Ruth Hill (see Chapter 21). Studies in mammalian cells began later, after cell culture techniques were refined, particularly by the work of Mortimer Elkind described in Chapter 21.

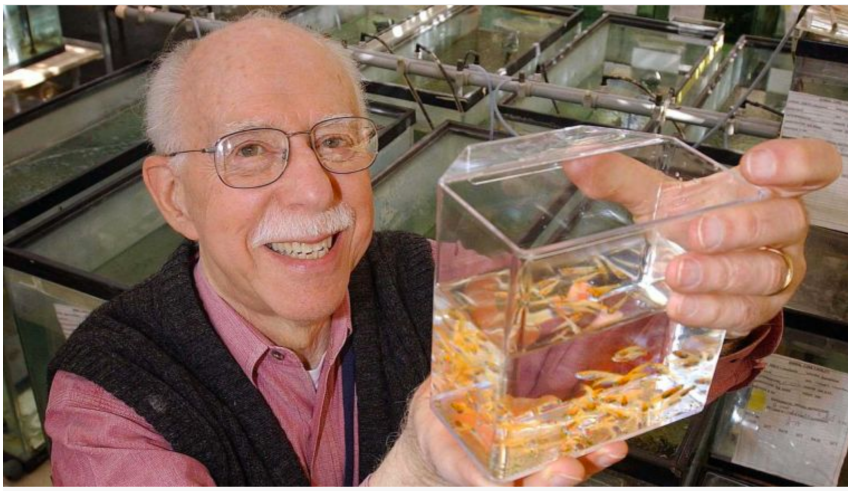

Figure 23.4. Richard ("Dick") Setlow (1921-2015) was a biophysicist at Oak Ridge National Laboratory in Tennessee, as well as an adjunct Professor at Stony Brook University on Long Island, New York, and a member of the National Academy of Science. He is best known for advancing the frontiers of what we know about how DNA damage is repaired. (Photo published in Long Island/Obituaries, provided by Setlow family.)

Nucleotide excision repair (NER): discovering how it works.

As Bernard Strauss noted not so long ago, NER works only because DNA is double-stranded, which allows a damaged region of one strand to be cut out and replaced by copying undamaged information from the undamaged complementary strand. He suggested that this duplication of information may be one reason why DNA is double-stranded (Strauss, 2018). Other types of life may be discovered thriving in other planets of our solar system under what would for us be noxious conditions; those alternative living systems would do well also to have chemical duplicates of their genetic information.

An early clue to the workings of NER, came from tracking down some peculiar differences among xeroderma pigmentosum (XP) cells of different complementation groups (see Chapter 22 for explanation of XP complementation groups and Table 23.1 or 22.2 for a list). In 1975, Ken Kraemer and his colleagues reported that cells defective in XPC retained the ability to respond to UV light albeit with a reduced rate of unscheduled DNA synthesis (see Chapter 22) (Kraemer et al., 1975a; Kraemer et al., 1975b). It seemed that there was a type of NER that did not require a normal XPC gene. Subsequent research disclosed two modes of NER: global-NER and transcription-coupled NER (TCNER), which came to light from studies of cells from the XP-related genetic disease, Cockayne's syndrome. A peculiar characteristic of those TCNER-deficient cells was that that they did not require the function of a normal XPC gene to carry out unscheduled DNA synthesis.

Global-NER repaired damage anywhere in the genome, while TCNER specialized in repairing damage in regions of the genome that were in the process of being transcribed. The proteins responsible for TCNER were found to be bound to the transcription complex as it moved along to transcribe the DNA. The molecular interactions involved in both types of repair are diagrammed in Figure 23.5 in the form of a molecular interaction map (Kohn, 1999).

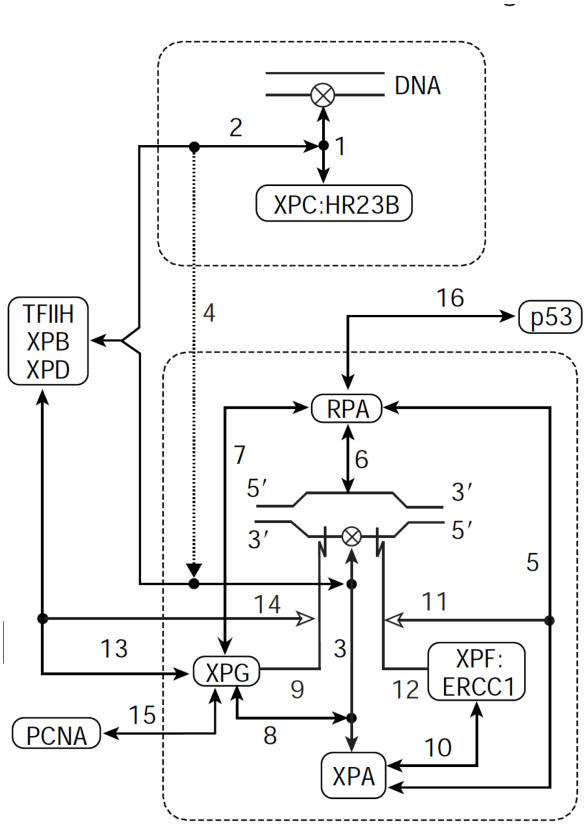

Figure 23.5. Molecular interaction map (Kohn (1999) of nucleotide excision repair (NER) in humans showing the roles of the XP proteins, as understood in 2001 (Kohn and Bohr, 2001). The meaning of the symbol for each step is explained in Box 1. Essentially: first, XPC (in complex with HR23B) detects and binds to the damaged site (shown as an x in a small circle). XPC then brings in XPB and XPD, which unwind the DNA helix on both sides of the damage. XPA then replaces XPC at the damage site. The XP's, together with RPA and some other proteins all bind together to form a big complex, which allow XPG to cut the strand at one side of the damage and XPF to cut it on the other side. That is how the damaged section of DNA is cut out. We see that the XP's, together with some other proteins, work together to cut out a section of damaged DNA strand. A DNA repair polymerase then comes along to extend the cut strand from its 3' cut end and copies the information in the complementary strand. Finally, a DNA ligase seals the newly replicated DNA to the 5' cut end of the strand. (The repair polymerase and ligase steps are straight-forward and are not included in the diagram.)

Figure 23.5 shows how the XP proteins work together in nucleotide excision repair. Global-NER must first detect and then cut the damaged region out of the damaged DNA strand. It must then replace the gap in the damaged strand with DNA copied from the undamaged strand. The mechanism whereby NER detects and cuts out the damage, as understood in 2001, is diagrammed in Figure 23.5. What happens at each step is explained in Box1.

The first challenge for global-NER is to find where the damage sites are located within the enormous length of the genome. The main actor to accomplishing this feat is the protein encoded by the XPC gene. The upper dashed box in Figure 23.5 depicts a normal double-stranded DNA helix bearing a lesion that is recognized and bound by a combination of XPC and HR23B. A DNA segment surrounding the lesion is then unwound by the XPB and XPD helicases, and XPA replaces XPC at the damage site (indicated by an x within a small circle). RPA, a single-strand binding protein, then binds to the undamaged DNA strand in the unwound region of the DNA helix.

The lower dashed box depicts the region of unwound helix and the cutting of the DNA single-strand segment on either side of the lesion by the endonucleases XPF-ERCC1 and XPG. The transition from the closed to the unwound state of the DNA (with associated proteins) is indicated by the long vertical dotted line, labelled interaction 4. The transition replaces the XPC:HR23B complex by XPA. XPA then assembles proteins that participate in the excision of the lesion.

In the case of transcription-coupled repair (TCNER), the repair proteins don't have to search for damage sites, because the repair machinery is bound to the transcription machinery and comes into play when the transcription encounters a damaged site. Moreover, the DNA helix around the lesion is already unwound due to the transcription process, and therefore repair can begin with XPA (lower box in Figure 23.5) and therefore does not need XPC to initiate the unwinding. That explains why normal XPC function is not required for TCNER.

Box 1. Explanation of the reactions/interactions of the steps numbered in Figure 23.5:

(1) A lesion in one strand of an intact DNA helix binds a dimer consisting of the XPC and HR23B proteins.
(2) XPC:HR23B binds the TFIIH transcription complex, which contains the DNA helicases XPB and XPD (Yokoi et al., 2000). All of these proteins are needed for the initial opening of the DNA helix at the site of the lesion. XPB and XPD on opposite sides of the lesion to unwind the DNA for a short distance on both sides of the lesion.
(3) XPA can then bind to the lesion (however, in the case of TCNER, the helix is already opened by the transcription process, and XPA can bind to the lesion without the aid of XPC:HR23B).
(4) The vertical hatched line with solid triangle arrowhead indicates that the DNA helix opens and XPC:HR23B is replaced by XPA. The TFIIH complex now is bound to XPA instead of to XPC:HR23B.
(5) XPA binds the DNA single-strand-binding protein RPA.
(6) RPA binds the undamaged strand where the helix has been opened. Thus, RPA helps to stabilize the XPA complex at the site of the lesion.
(7) RPA recruits endonuclease XPG.
(8) XPG binds XPA, which is bound to the lesion.
(9) XPG cuts the lesion-containing strand on the 3' side of the lesion.
(10) XPA recruits the XPF:ERCC1 heterodimer to the lesion site.
(11) RPA binds XPF and directs it to cut on the 5' side of the lesion.
(12) XPF cuts the lesion-containing strand on the 5' side, thereby releasing a segment of DNA single-strand containing the lesion.
(13) TFIIH binds to XPG.
(14) TFIIH positions XPG on the 3' side of the lesion.
(15) XPG recruits PCNA, which is required for the subsequent DNA repair synthesis that fills the gap left by the excised single-strand segment.
(16) RPA binds p53 and thereby signal the presence of DNA damage.

XPF-ERCC1 as a chemotherapy target.

In the late 1970's and early 1980's, many genes were identified that corrected DNA-repair defects in human and rodent cells. In addition to the XP genes, several other DNA repair-correcting genes were identified, among which there was a gene that came to be called ERCC1. This ERCC1 gene was cloned in 1984 (Westerveld and Naylor, 1984), and in 1993 it was found to correct the defect in XPF cell lines (Biggerstaff et al., 1993). The XPF and ERCC1 proteins were then found to bind tightly to each other. The XPF-ERCC1 complex constituted the enzyme that cuts the DNA strand on the 5' side of the lesion. Looking at Figure 23.5, we see that XPF-ERCC1 functions in NER to cleave the damaged strand on the 5' site of the damage site (reaction 12 in Figure 23.5). Moreover, the XPF-ERCC1 complex

also has a similar role in other DNA repair pathways, such as in the repair of DNA interstrand crosslinks. In general, it cleaves a DNA strand where a region of unwound DNA joins normal duplex DNA, as we see in Figure 23.5.

ERCC1 attracted attention when high ERCC1 expression was found to favor the long-term survival of lung cancer patients whose tumors were resected by surgery without chemotherapy (Simon et al., 2005) (Figure 23.6). On the other hand, high ERCC1 expression *reduced* the survival in advanced non-small cell lung cancer (NSCLC) treated with chemotherapy (Lord et al., 2002) (Postel-Vinay et al., 2012) (Figure 23.7). Although the circumstances of the two studies were quite different, they suggested that ERCC1 had two-faces when it came to whether it benefits or harms. It was presumed that high ERCC1 level would increase DNA repair activity. Lung cancer patients whose tumors were caught early enough to be amenable to surgery without chemotherapy benefited if their tumors expressed ERCC1 at a high level. The benefit would have been due to enhanced DNA repair when there was lots of ERCC1, which would reduce the DNA damage that happens because of the unrestrained division of cancer cells. On the other hand, the detrimental effect of high ERCC1 would come from the consequent high DNA repair activity countering the DNA damage whereby chemotherapy kills cancer cells. Hence, high DNA repair capacity could counter the therapeutic action agents, such as cisplatin, whose therapeutic action depends on producing DNA damage. Therefore, inhibitors of XPF-ERCC1 functions were recently developed and were found to synergize with the DNA damaging agents, cisplatin and mitomycin, in killing cancer cells culture (Ciniero et al., 2021).

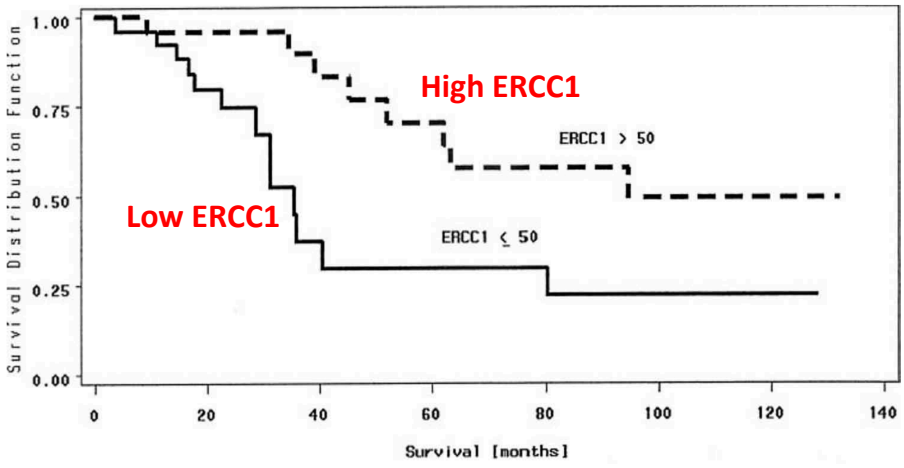

Figure 23.6. Lung cancer (NSCLC) patients whose tumors were localized enough to be resected by surgery, had a better chance of long-term survival if their cancer expressed ERCC1 at a high level (Simon et al., 2005).

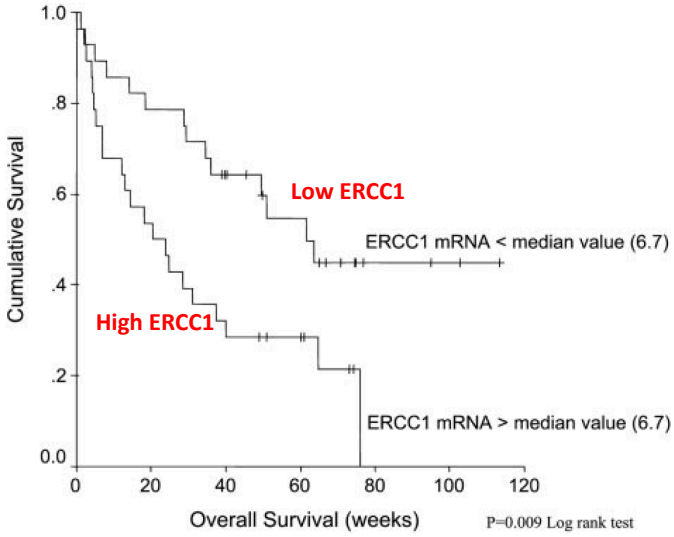

Figure 23.7. Advanced lung cancer (NSCLC) patients who were treated with a combination of cisplatin and gemcitabine survived longer if their cancer's ERCC1 expression was *low* (Lord et al., 2002).

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