Chapter 21. The DNA Repair Story: early discoveries 220724gc3

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

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CHAPTER 21

The DNA Repair Story: early discoveries.

Introduction

DNA damage from metabolic and environmental sources is unavoidable; cells therefore have evolved an astonishing set of mechanisms to counter the great variety of chemical damage that the cell's DNA can sustain. Defects in one or another DNA repair mechanism often lead to cancer, but cancer cells whose DNA repair mechanisms are defective or inefficient are vulnerable to DNA-damaging anti-cancer drugs.

Much about DNA damage and repair first came to light from studies of how bacteria do it. How our cells (or mammalian cells in general) do it, first came from studies of cells in culture, where studies could be carried out under precisely controlled conditions. Cell culture studies then unraveled much of the mystery of what anticancer drugs do to cells and how the cells respond.

A specific DNA repair mechanism was already encountered and described in Chapter 2, namely the removal of drug adducts at the O6-position of guanines by methylguaninemethyltransferase (MGMT). But even that highly efficient repair process has limits – no biological process is perfect. Backup processes are therefore needed to clean up any adduct that may be left unrepaired.

A normal dividing cell's DNA is constantly at risk of being damaged by environmental carcinogens or radiation, or by the rare but inevitable errors made by the machinery that normally replicates DNA. Many types of DNA damage are produced, and their frequencies of occurrence vary greatly (Table 1). It must have challenged evolution to create the collection of DNA repair

mechanisms to deal with the many different kinds of chemical damage that DNA can suffer (Figure 21.1) (Lindahl, 1982; Sancar and Sancar, 1988). Most cancer chemotherapeutic agents, including drugs and radiation, damage DNA in one way or another, and DNA repair mechanisms come into play to cope with the various kinds of damage.

Cancer cells often are deficient in one or more DNA repair processes and then may become sensitive to drugs producing types of damage that the cells of the cancer are unable to repair. More generally, cancer cells often have defects in regulatory mechanisms, including those that govern responses to the genomic stress caused by DNA damage. These defects may impair the cancer cell's ability to cope with the drug-induced damage, for example by increasing the capacity of the repair machinery or by delaying DNA replication or mitosis to give more time for repair.

Even though the cell has many different molecular repair machines, as well as control networks to give more time for repair, a small amount of damage inevitably gets through (Gudmundsdottir and Ashworth, 2006). The reason anticancer drugs work is often because the defects in cancer cells may allow more unrepaired damage to remain when the DNA begins to replicate or the cell begins to divide, which is when persisting DNA damage would be apt to kill the cell.

Cancer cells often have DNA repair defects that make them susceptible to DNA-damaging anticancer drugs. Another kind of defect that often makes cancer cells susceptible is a defect in a "cell cycle checkpoint." These checkpoints consist of molecular circuitry that check whether it is safe for the cell to proceed from one phase of the cell cycle to the next. Unrepaired DNA damage triggers the checkpoint circuitry to delays the cell cycle in order to give more time for repair before the cell is allowed to progress to the next phase where persistent DNA damage could result in cell death.

As impressive as our DNA repair armamentarium may be, we mammals are far from champions in that field. Far more impressive among animals are the tiny tardigrades and some even tinier rotifers (Figure 21.2), which can survive hundreds of times as much DNA damage than our cells could tolerate. In the course of their evolution, these creatures have acquired, by means of gene transfer, a large collection of DNA repair genes from various species of eukaryotes and bacteria. This helps them survive for years dried-out in a desiccated state in which they are subject to extensive DNA damage (Hashimoto and Kunieda, 2017). This seeming bit of trivia about DNA repair proficiency might eventually be matched by humans, if gene transfer technology became feasible and ethically acceptable: inserting extra DNA repair genes might extend lifespan and allow astronauts to survive (albeit not in a desiccated state) the radiation in space and extraterrestrial planets, moons and asteroids. The potential impact on cancer therapy is difficult to

predict but may be significant.

Table 1 Estimated frequencies of DNA lesions normally occurring in mammalian cells

Damage	Events per cell per day
Single-strand breaks	55 000
Depurinations	13 000
Depyrimidinations	650
Guanine-O6 methylation	3100
Cytosine deamination	200
Glucose-6-phosphate adduct	3
Thymine glycol	270
Thymidine glycol	70
Hydroxymethyluracil	620
Guanine-8 oxygenation	180
Interstrand cross-link	8
Double-strand break	9
DNA-protein cross-link	Unknown

From (Kohn and Bohr, 2001).

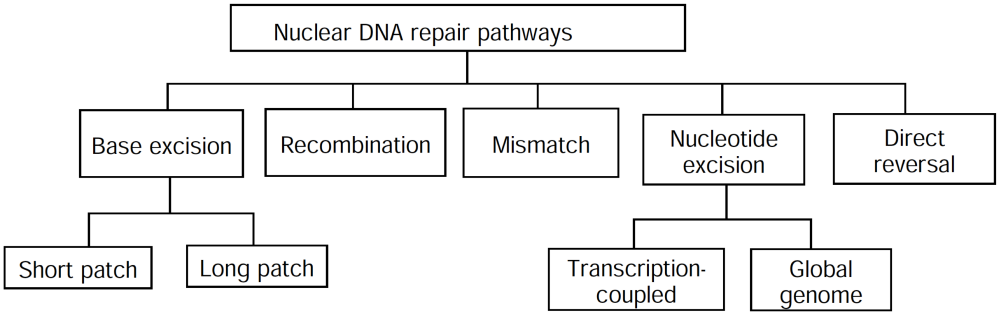

Figure 21.1. An overview of the types of DNA repair mechanisms operating in the cell nucleus. *From (Kohn and Bohr, 2001)*.

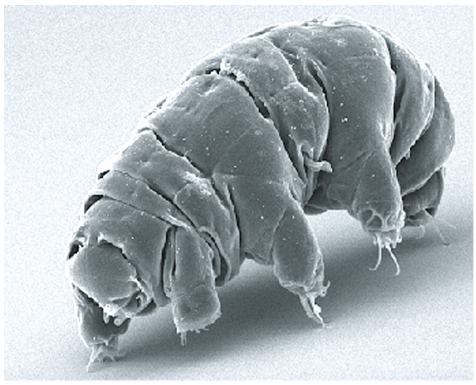

Figure 21.2. Scanning electron microscope image of a tardigrade: a champion DNA repairer.

Discovery of DNA repair.

Discovery of DNA and its structure

Before talking about the repair of DNA damage, I'd like to talk about how the genetic material was found to be made up of – well, DNA. But first I am reminded of Edwin Chargaff, discoverer of the DNA base-pairing rules (G=C and A=T), whom I encountered in 1953 during my first year at Columbia's medical school, the College of Physicians and Surgeons (P&S), when he conducted biochemistry laboratory classes. On asking him about -- admittedly wild -- ideas I had, he dismissed them out of hand; he certainly was not encouraging. My future wife, Elaine Kay Mogels, worked in a research lab at P&S and attended Chargaff's course on nucleic acids, but she didn't find it very interesting. We had his 2-volume work "The Nucleic Acids" but I did not refer to it much during the coming years, because my projects did not require the early chemistry details that it focused on.

I also recall Chargaff's lecture at a molecular biology conference at Columbia in 1960 – I think it was – in which he denigrated the papers in the new molecular biology field that were coming out, even if published in journals like the Proceedings of the National Academy of Sciences, that he said were akin to what he might read in the New York Times. He thought it soft science, not up to par with the solid previous work on nucleic acids. In that regard, he extoled particularly the work of Johannes Miescher.

Johannes Friedrich Miescher (Figure 21.3) wanted to find out what the cell nucleus was made of, about which next to nothing was known in 1868, when at the age of 24 he came to the University of Tubigen, Germany, to study with Ernst Hoppe-Seyler, a founder of the new field of biochemistry. In 1869, Miescher isolated from cell nuclei a strange highly viscous phosphate-rich material that he called "nuclein" --which was in fact DNA with some bound

protein. He had a hunch that the large molecules in his nuclein might be the genetic material, an idea that he expressed in a vaguely worded letter to his uncle in 1892, but this idea lay dormant for decades (Judson, 1979).

Over the next three decades after Miescher's extraction of "nuclein" from cell nuclei, hard work by many researchers disclosed the chemical structures of the DNA constituents guanine, adenine, thymine and cytosine, and DNA was found to be made up of long chains of deoxyribose-phosphate bound to one or another of those 4 bases, which made up the four nucleotides: G, A, T, and C. A wrong idea emerged that DNA was a polymer of unvarying groups the 4 nucleotides. This "tetranucleotide hypothesis" implied boring tetranucleotide repeats that could not possibly be the chemical composition of genes. For a long time, many biochemists dismissed DNA as likely having a structural role and held that genes must be made up of proteins, which had complex structures that they thought to be commensurate with the complexity of genetic information.

The controversy about the chemical basis of genes continued despite mounting evidence favoring DNA until 1944 with the definitive experiments by Oswald T. Avery (Figure 21.4) (Avery et al., 1944). Avery and his colleagues at the Rockefeller Institute in New York were studying peculiar phenomena that had first been observed by Frederick Griffith in 1928 in the pneumonia-inducing *pneumococcus* bacteria.

Griffith's experiment was a bit complicated, but here goes: Each bacterial cell was able to grow into a colony on an agar surface. The colonies sometimes had a smooth appearance and sometimes a rough appearance, depending on the bacterial strain. Bacterial strains were called S or R, depending on whether they grew into smooth or rough colonies. S-strain pneumococci produced pneumonia in mice, whereas R-strains did not. There were 3 types of *pneumococcus*, called types I, II, and III; each type had S and R strains that usually bred true to their particular strain. Injecting mice with an R strain of type II did not produce pneumonia, but – surprise! – R of type II mixed with *heat killed* S of type III did produce pneumonia! Moreover, some of the R type II apparently had *transformed* to behave like S type III!! It turned out that the transformation could be made to happen merely by incubating the two kinds of bacteria together in a nutrient-deficient broth. A genetic characteristic – indeed, a gene or genes – were being transferred from one bacterial cell to another.

What Avery and his colleagues did was show that the gene transfer occurred when *pure* DNA from donor bacteria was mixed with recipient bacteria. They did many checking experiments to support their contention that the genetic information was in the DNA and not in a protein impurity. Some researchers nevertheless persisted for years thinking that an undetected trace of a protein

impurity, impervious to Avery's protein-digesting enzyme test, was the holder of the genes. A fond idea long held was difficult to discard.

The phenomenon of genetic transformation was later found in several bacterial species. But it occurred only when the recipient bacteria were *competent* to take up the donor DNA. What made bacteria competent became a question of intense investigation. As a post-doc in Paul Doty's laboratory at Harvard, I collaborated with Donald MacDonald Green using DNA transformation in *Bacillus subtilis* to show that a single nitrogen mustard-induced inter-strand crosslink abolished the DNA's transforming ability (Kohn and Green, 1966) (see Chapter 1).

Successful genetic transformation of mammalian cells by DNA was first reported by Waclaw Szybalski (Szybalska and Szybalski, 1962) but was very difficult to reproduce consistently. The story of how modification of technique eventually made it an essential procedure in modern cell biology is told in Chapter 15.

It was only a few years after DNA was accepted to be the bearer of genetic information that Watson and Crick presented a correct model of DNA structure (Figure 21.6).

Figure 21.3. Johannes Friedrich Miescher (1844-1895) was one of the great biological chemists of his time. He received an MD degree from the medical school at Basel, Switzerland in 1868 and then joined Felix Hoppe-Seyler's laboratory at the University of Tübingen, Germany, to investigate the constituents in cell nuclei. He succeeded in extracting from cell nuclei a highly viscous phosphate-rich material that he called nuclein, which was later found to be composed mostly of DNA. Because of its high viscosity, Miescher correctly concluded that his nuclein was made up of very long molecules, and he suspected it to be the genetic material, an idea that was dismissed for decades before it was shown to be true.

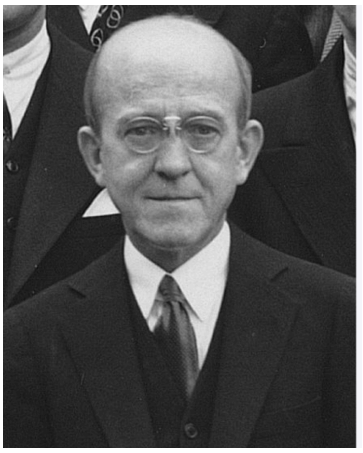

Figure 21.4. Oswald Theodore Avery, Jr. (1877-1955) and his colleagues at the Rockefeller Institute in New York purified DNA and proved that it contained genetic information. His definitive experiments, published in 1944, became a famous landmark that propelled later studies of DNA and genetics.

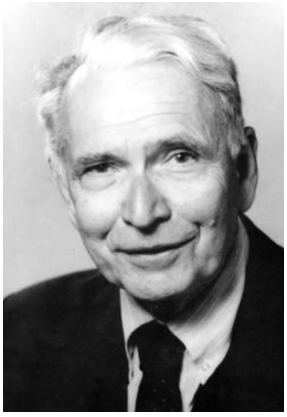

Figure 21.5. Erwin Chargaff (1905-2002) was professor of biochemistry at Columbia College of Physicians and Surgeons (P&S) from 1938 to 1970 and served as chair of the department until his retirement in 1974. His family had moved to Vienna in 1914 from their home in Czernowitz, now Chernivtsi, Ukraine. He attended the Vienna *Technische Hochschule* and earned a PhD in chemistry from the University of Vienna in 1928. He became renowned for his discovery that the frequencies of the nucleotides (G, C, A, T) in DNA, while differing among various organism, had a remarkable pattern that the frequencies of G and C tended to be equal, as was the case also for A and T. This “Chargaff rule” was later found to reflect the base-pairing in the DNA double-helix (G=C and A=T).

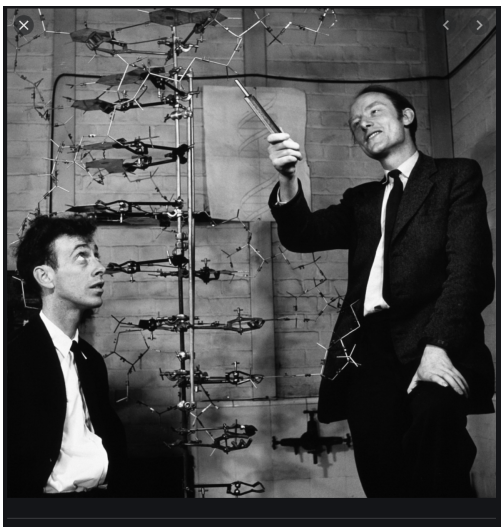

Figure 21.6. James D. Watson (left) and Francis Crick (right) with their DNA model circa 1952.

How DNA repair was discovered.

When DNA is damaged by radiation or chemicals, the cell can usually repair the resulting mutation. I will begin by looking back at how this understanding developed. A key discovery about mutation of genes was made by Hermann J. Muller in 1927 (Figure 21.7), for which he was awarded the Nobel Prize in Physiology or Medicine in 1946. Muller had been studying hereditary changes in fruit flies and discovered that x-rays produced mutations in proportion to the x-ray dose. That finding, in a sense, initiated the field of DNA damage (even though it was not yet known that DNA was the genetic material) (Friedberg, 1997). The mutation story however dates back even further, to Charles Darwin's evidence, published in *Origin of Species* on 24 November 1859. His evidence implied that hereditary changes in species were due to "mutation" of genes (although the term "mutation" was not introduced until the late 1880's).

Long before DNA became known to be the genetic material, it was already known that genes were lined up in chromosomes and that x-rays or ultraviolet light caused chromosome breaks resulting in mutations (Goldschmidt, 1951). The ends of broken chromosomes were sticky and could join up, the end of one chromosome break becoming joined to the end of a different chromosome break (McClintock, 1951) (Figures 21.8 and 21.9.). The chromosome breaks were thereby repaired, but at the cost of changes in the lineup of the genes, and, if a break occurred within a gene, the function of the gene was destroyed.

Figure 21.7. Hermann Joseph Muller (1890-1967) with his x-ray machine. Muller entered Columbia College in New York at age 16, where he developed a long-time concern for the relationship between biology and society and became a proponent of eugenics. After completing his PhD degree at Columbia, Muller joined Thomas Hunt Morgan's "Fly Room" at Columbia where fruit fly genetics was under intense investigation, for which Morgan was awarded a Nobel Prize in Physiology and Medicine in 1933. Muller was awarded a Nobel Prize in Physiology and Medicine in 1946 for his discovery that x-rays cause mutations and quantifying the effect.

Figure 21.8. Barbara McClintock (1902-1992) discovered mobile genetic elements, a concept so revolutionary at the time that it was long before geneticists accepted or understood it. Many years later after the importance of her of her discovery was grasped, she was awarded a Nobel Prize in Physiology or Medicine in 1983. She had received a PhD in Botany at Cornell University in 1927 and used maize as her subject of investigations.

(From http://siarchives.si.edu/collections/siris_arc_306310)

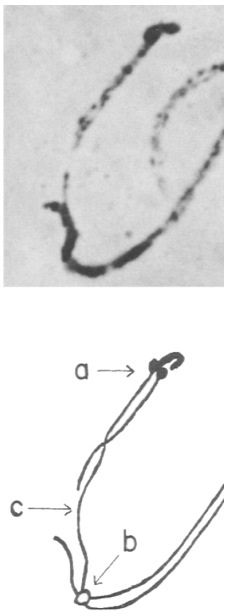

Figure 21.9. An example of how Barbara McClintock observed chromosome breaks and their consequences (McClintock, 1951). The two homologous chromosomes are joined during mitosis at their centromeres (b); one of the chromosomes of the pair is broken (c).

The first evidence that the damage caused by ultraviolet light (UV) or x-ray could undergo some kind of repair, came from experiments with bacteria. When E. coli bacteria were irradiated, the individual bacteria lost their ability to grow into colonies. However, if the irradiated bacteria were held for a period of time in a medium that lacked ingredients needed by the bacteria to grow and then put back into their growth medium, they recovered some of their ability to grow into colonies (Roberts and Aldous, 1949) (Harm, 1966). That remarkable observation suggested to the researchers that some kind of repair was happening. (The interpretation of those early experiments became complicated by a later discovery that photoreactivation, a process wherein the UV light itself stimulated an enzyme that removed some of damage. However, the broth in which the irradiated bacteria were held may have absorbed enough light so that photoreactivation was insignificant. Our body cells, by the way, do not have the ability to photoreactivate UV-induced DNA damage. That is one of the many metabolic abilities that microbes have that we lack (or that humans might some-day recover by gene transfer, if that were ever to become permitted.)

Another important early discovery was of a strain of bacteria, called E. coli B/r, that was much less sensitive to DNA damage by ultraviolet light or x-ray than its parent strain, E. coli B (Witkin, 1946) (Figure 21.10). Although Evelyn Witkin, who discovered the resistant strain in 1946, knew little yet about DNA, her B/r strain became an important tool in DNA damage and repair studies. Then in 1958, R. F. Hill isolated a hypersensitive mutant called Bs1, which joined the B/r strain to become mainstays of DNA damage and repair studies (Hill, 1958).

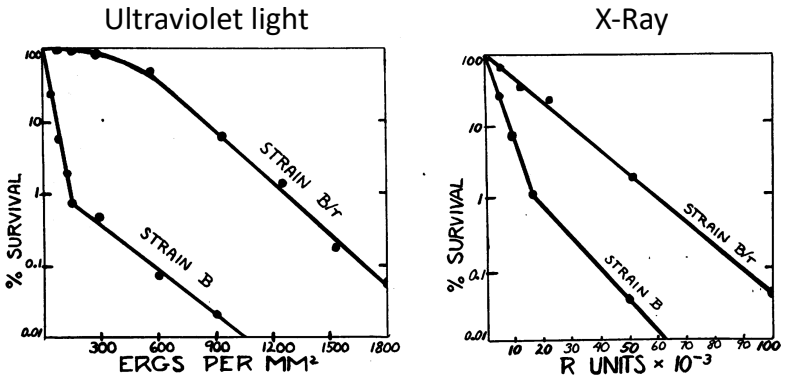

Figure 21.10. Evelyn Witkin's discovery in 1946 of a strain of E. coli bacteria, called B/r, that was resistant to ultraviolet light and x-rays, compared to its parental strain, E. coli B (Witkin, 1946).

First evidence of DNA damage repair in mammalian cells.

A major figure in the early studies of DNA damage and repair in mammalian cells was Mortimer Elkind at the National Cancer Institute and then at the Argonne National Laboratory (Figure 21.11). Elkind demanded rigorous quantitative discipline in his research and put the relationships between DNA damage, repair, and cell survival on a sound basis. He proved that unrepaired DNA damage caused cells to die (Elkind, 1979) -- which resulted in DNA damage and repair becoming a focus of expanded research in many laboratories on how anticancer drugs kill cancer cells.

Elkind's precise quantitative methods were already evident in his first major publication in this field (Figures 21.12 and 21.13) (Elkind and Sutton, 1960). Figure 21.12 shows how Elkind and Sutton quantified the killing of cells by x-rays. In these kinds of experiments, it was important to choose the best materials to work with. The cells chosen had to grow well on the surface of a glass dish, and they had to have a consistent growth curve (number of cells versus incubation time). Elkind used a cell line that fit that requirement well: a clone of fibroblasts from Chinese hamster cells, called V79. After being

incubated for a suitable number of days, each viable cell on the dish formed a colony, and the number of colonies could be counted. The survival of cells after a given dose of x-rays was gaged as the fraction of cells that retained the ability to form colonies.

Elkind and Sutton used that quantitative method to study the relationship between colony survival and x-ray dose. The experiment in Figure 21.13 is marked as number 92, showing the thorough persistence in carrying out this labor-intensive work. The precision of their data, together with the mathematical theory they derived, gave the first indication that mammalian cells had the ability, although limited in extent, to repair the damage caused by x-rays. Moreover, the linear part of the survival curves indicated that a single unrepaired radiation-induced damage event could kill a cell (Elkind, 1984).

The P.E. (plating efficiency of individual cells) in the experiment shown in Figure 21.13 was marked 84.1%, meaning that 84.1% of the unirradiated cells put on the plate grew into colonies. In later experiments, the plating efficiency was even higher. A high plating efficiency was important for quantitative interpretation of the data. Low plating efficiencies of individual cancer cells later came to haunt us when our Developmental Therapeutics Program tried to use colony forming ability of cells extracted directly from human cancers to look for drugs that would work against the common solid tumors: the apparent plating efficiencies in those attempts was miniscule! After several years of effort and large investment of resources, the project had to be dropped – all because the tiny plating efficiencies of individual cancer cells were overwhelmed by cell clumps. This instructive fiasco was related in Chapter 20.

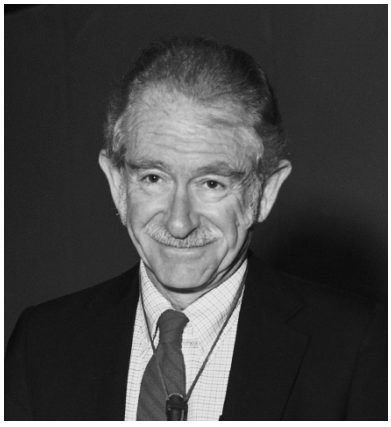

Figure 21.11. Mortimer (Mort) Elkind (1922-2000) was one of the greats of Radiation Biology and quantitative cell culture studies, which he carried out over many years in the National Cancer Institute. He pioneered the precise quantitation of cell killing by radiation and anti-cancer drugs. Originally trained in engineering and physics, he applied the strict discipline of those fields to cell biology (Withers, 2003).

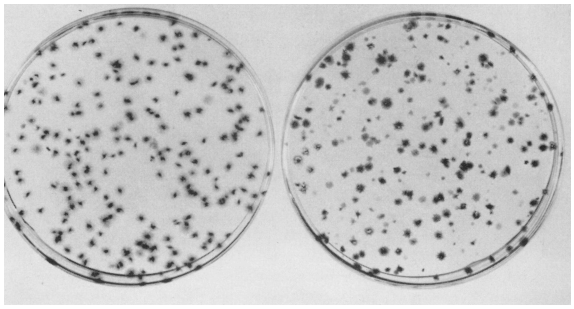

Figure 21.12. Quantitative determination of the killing of mammalian cell by x-rays. *Left*, no radiation; *Right,* 542 rad of x-rays. The cells used were from Chinese hamsters and grew on the surface of a glass dish. A single cell could divide and form a colony that was then made visible by means of a stain. The colonies were counted in order to determine the fraction of the cells that survived to form colonies after a given dose of x-rays (Elkind and Sutton, 1960).

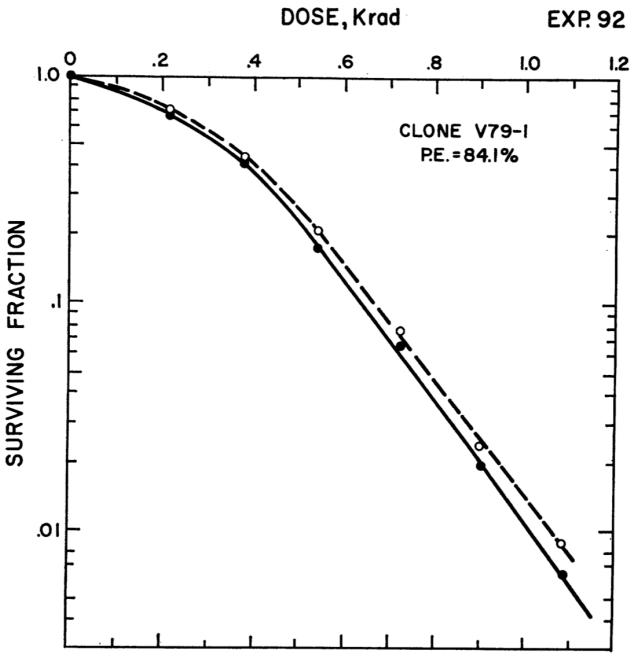

Figure 21.13. An x-ray survival curve with a "shoulder" at low doses (below 0.4 krad in the Figure). This was the first indication of repair of x-ray-induced damage in mammalian cells (Elkind and Sutton, 1960). Cells were grown on the glass surface of a dish, as described in Figure 21.12, and the survival of colony-forming ability was determined by counting the number of colonies formed before and after various doses of x-ray. The shape of the survival curve, including the linear portion in this semi-logarithmic plot fit a

mathematical theory derived by Elkind and Sutton and presented in their 1960 paper.

DNA damage and repair investigated at the molecular level.

The year 1960 was a harbinger of things to come in the field of DNA damage and repair. The first clues to the chemistry of DNA damage came from two landmark discoveries, both reported in 1960 (Brookes and Lawley, 1960) (Beukers and Berends, 1960). Peter Brookes and Philip Lawley at the Chester Beatty Cancer Institute in London treated various sources of DNA and RNA, as well as a tumor in mice, with sulfur mustard having a radioactive sulfur isotope. Analyzing the treated DNA and RNA for altered nucleotides they found one dominant product: the major part of the sulfur mustard molecule became bound to guanines at the N7 position (Figure 21.14) (Brookes and Lawley, 1960). To honor their achievements, the UK in 2003 established the Brookes-Lawley Laboratory for research on the genetic nature of cancer, as part of the Institute for Cancer Research (ICR) in London. Further studies described in Chapter 1, including those I too began in 1960, showed that this reaction was the first step in the production of a DNA inter-strand crosslink (Kohn et al., 1966).

Also in 1960, Dutch researchers R. Beukers and W. Berends, working in the Biochemical and Biochemical Laboratory of the Technological Institute of Delft, The Netherlands, showed that ultraviolet light caused thymine (in frozen solution) to link in pairs to form dimers (Figure 21.15) (Beukers and Berends, 1960) (Beukers et al., 2008). The significance of this discovery soon became evident.

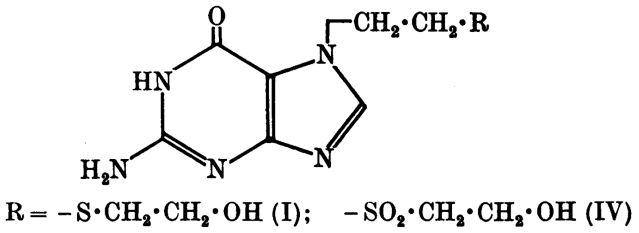

Figure 21.14. The chemical structure of the adduct at the N7 position of guanine that Peter Brookes and Philip Lawley identified in DNA and RNA treated with sulfur mustard (Brookes and Lawley, 1960) (the chemistry of sulfur and nitrogen mustards was discussed in Chapter 1).

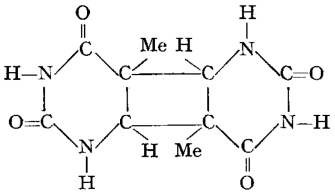

Figure 21.15. *Left*, the General Electric germicidal low-pressure mercury vapor ultraviolet lamp used by Beukers and Berends in 1960 to create thymine dimers by irradiating a frozen aqueous solution of thymine in the small dish shown (Beukers et al., 2008). *Right*, the chemical structure of the thymine dimer, which they correctly inferred from infrared spectra that suggested the presence a cyclobutene (4-membered) ring (Beukers and Berends, 1960).

In 1963, Richard Setlow and his colleagues at Oak Ridge National Laboratory in Tennessee made a landmark discovery that initiated the long and tortuous history of DNA damage repair studies. Their experiments showed that E. coli bacteria were able to repair the DNA damage produced by ultraviolet light (UV). When they irradiated bacteria with ultraviolet light (UV, 265 nm), thymine dimers were produced similar to those reported in 1960 by Beukers and Berends in UV-irradiated frozen solutions of thymine. Setlow inferred that UV-induced thymine dimers in the DNA inhibited the ability of the bacteria to synthesize DNA and to grow to form colonies (Setlow et al., 1963).

Investigating further, they compared the UV-sensitive strain, E. coli Bs1, with the resistant strain, E. coli B/r, for the abilities of the bacteria to recover DNA synthesis after exposure to UV light (Setlow et al., 1963). They found that E. coli B/r was able to remove thymine dimers from DNA, whereas E. coli Bs1 was defective in this ability. After removal (repair) of the thymine dimers, the bacteria recovered their ability to synthesize DNA (Setlow and Carrier, 1964) (Figure 21.16). This was the first evidence for the existence of a DNA repair mechanism that came to be called *nucleotide excision repair* (Chapter 23) (Setlow et al., 1963).

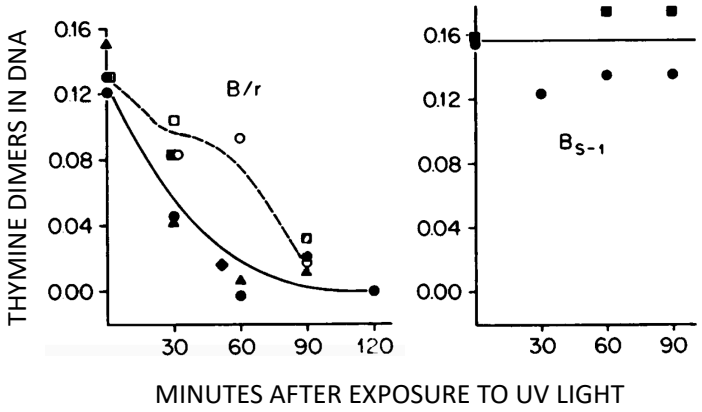

Figure 21.16. The first demonstration of DNA repair in living cells ((Setlow and Carrier, 1964), modified). E. coli bacteria were exposed to ultraviolet light (UV), and the thymine dimer content of the DNA was then measured after various lengths of time. The UV-resistant variant (B/r, left) removed dimers with time, whereas the UV-sensitive variant (B_{s1}, right) did not. (The upper curve in the B/r experiment was when the incubation after UV was in medium lacking an energy source (glucose); in all other experiments, the incubation was in complete medium.)

Next, in 1969, Setlow, Carrier and their colleagues found that normal human cells, like the bacteria, have the ability to remove thymine dimers from their DNA. Additionally, however, another landmark discovery was that cells from patients with the genetic disease xeroderma pigmentosum (XP) were unable to do that (Chapter 23). It seemed that normal cells could cut out the thymine-dimer damage from their DNA. The inability of XP patients' cells to carry out this DNA repair caused patients with this inherited disease to be extremely sensitive to daylight. Those early discoveries have been copiously summarized by Jim Cleaver (Cleaver, 2003) and by Errol Friedberg (Friedberg, 2011).

First evidence of repair of DNA of inter-strand crosslinks.

My involvement in the DNA repair story began when I started as a post-doctoral fellow in Paul Doty's laboratory at Harvard in 1959. I was looking for ways to create chemical crosslinks between the paired stands of DNA. I had most in mind nitrogen mustard as a possible DNA inter-strand crosslinking agent that might relate to its therapeutic capabilities. How it happened that I came to suspect that nitrogen mustard produced DNA interstrand crosslinks was told in Chapter 1. I discussed my idea about DNA interstrand crosslinking,

which was a new idea at the time, with Professor Doty and we considered various ways in which DNA interstrand crosslinks could be produced. Remarkably, all of the possibilities we considered were soon discovered independently by various investigators. It was evidently a case that, when concepts and methods become available, a field is ripe for rapid discoveries.

During our discussions in the laboratory, a possible way to produce DNA crosslinks was brought to my attention by K. Leszek Wierzchowski, a visiting scientist from Poland who had coauthored an extensive review article with David Shugar about the effects of ultraviolet light (UV), published in an obscure Polish journal. Lech told me about the just then published reports by Beuker and Berends that UV caused thymine to link in pairs to form dimers. We thought that UV might link together thymines in opposite DNA strands to form interstrand crosslinks. This turned out to be true, although most of the thymine dimers were later found to form between neighboring thymines in the same strand. However, the path to the discovery of DNA repair progressed when Setlow and his colleagues demonstrated UV-generated thymine dimers in bacteria, described above and in the Chapter that follows. My focus, however, was on the nitrogen mustard crosslinking idea, which I was enabled to pursue based on the helix-coil transition phenomena and theory that had been developed by Paul Doty, Julius Marmur, and others in his lab.

The main instrument I used in the nitrogen mustard experiments was the analytical centrifuge, an extraordinary technology which I soon managed to master with initial guidance by Carl Schildkraut, who was at that time an advanced graduate student in the lab. All of this is described in greater detail in Chapter 1, which tells how it happened that nitrogen mustard, a close relative of mustard gas, became the first chemical agent that shrank solid tumors in humans, and how my notion of crosslink production emerged.

After completing the nitrogen mustard crosslinking studies in physical chemical systems (Kohn et al., 1966), I returned to the National Cancer Institute in Bethesda with the aim of extending those studies to living organisms. I was convinced that nitrogen mustard worked in cancer chemotherapy by producing DNA crosslinks in cancer cells. The methods to prove that required development of new technology, which only became feasible years later after we developed DNA filter elution methods capable to measuring several types of DNA damage in mammalian cells (Chapter 9).

In the 1960's however we were able to test our ideas in bacteria, namely the DNA repair proficient and deficient strains, E. coli B_{s1} and E. coli B/r, respectively, and Setlow's findings using those strains, as described above. As expected, we found that the radiation-sensitive mutant, E. coli B_{s1}, was unable to repair DNA inter-strand crosslinks produced by nitrogen mustard, whereas the resistant E. coli B/r removed the crosslinks with high efficiency (Figure 21.17) (Kohn et al., 1965). This was the first evidence for repair of interstrand crosslink repair in living organisms.

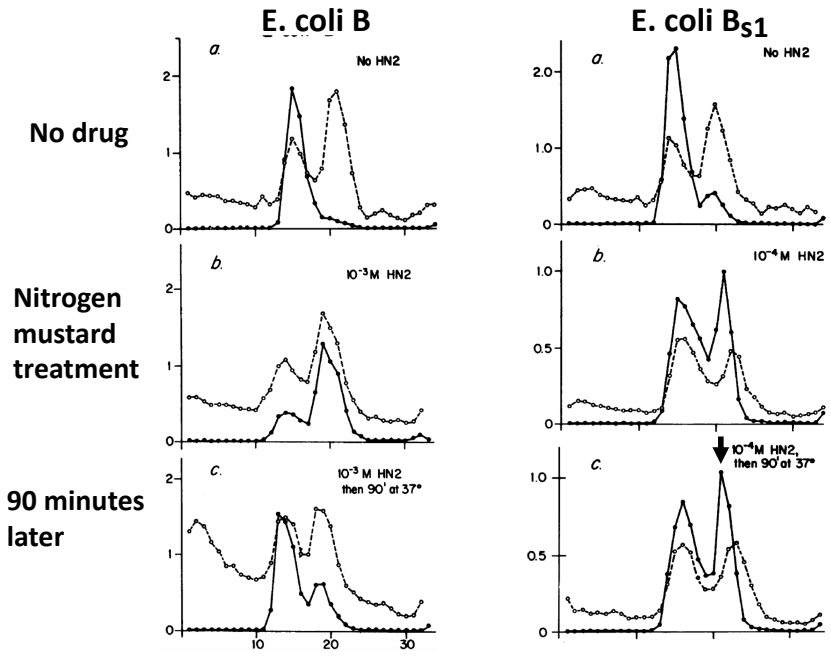

Figure 21.17. The first demonstration of DNA crosslink repair (Kohn et al., 1965). In this experiment, we found that wild-type E. coli B removed nitrogen mustard crosslinks from DNA, but that the radiation-sensitive variant, E. coli B_{s1}, was unable to do so. The peak representing the unrepaired crosslinks in E. coli B_{s1} is indicated by the arrow in the panel on the lower right. The DNA from the bacteria was radioactively labeled and is represented by the solid lines; the left and right peaks are non-crosslinked and crosslinked DNA, respectively. The DNA from the bacteria was treated with sodium hydroxide to separate the strands; after neutralizing the solution with citric acid, only the crosslinked DNA recovered its double-stranded form and banded in the peak on the right. The non-crosslinked DNA remained single-stranded and banded on the left. (The dashed curves show where the single-stranded DNA (left peak) and double-stranded DNA (right peak) would band.) The samples were ultracentrifuged in a concentrated CsCl solution for 60 hours to reveal the banding patterns shown.

DNA in the cell nucleus can be damaged in many ways: carcinogens in the environment, workplace, and foods; ultraviolet light from the sun; radiation from diagnostic x-rays, power plants, and natural background; chemotherapy drugs; normal metabolism and genetic defects. These produce a wide variety of chemical damage to DNA, for which an amazing variety of repair mechanisms have evolved. It is remarkable how evolution has come up machinery to fix or cope with almost any kind of DNA damage. Even when some damage remains, there are fail-safe mechanisms to cope with it. It seems that almost nothing will permanently stop a cell, except when the insult is overwhelming. Those issues are of great importance in cancer treatment. The ability of cancer patients to survive chemotherapy, depends greatly and the ability of normal tissues to repair the DNA damage caused by nearly all of those drugs. Moreover, the effectiveness of many of those drugs depends in part on the DNA damage they produce and the difficulty that cancer cells have in trying to repair them.

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