Genetic diseases reveal DNA nucleotide excision repair 220725ax3

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

Kurt W. Kohn, MD, PhD
Scientist Emeritus
Laboratory of Molecular Pharmacology
Developmental Therapeutics Branch
National Cancer Institute
Bethesda, Maryland
kohnk@nih.gov

CHAPTER 22

Genetic diseases reveal DNA nucleotide excision repair.

Rare inherited diseases reveal DNA repair genes and how cells cope.

It is remarkable how studies of certain rare inherited diseases gave clues that helped unravel the complexities of DNA damage repair, the factors that predispose to cancer, and the molecular targets for drug treatments tailored for the molecular defects in individual cancers. Table 22.1 lists genetic diseases that led to the discovery of genes for the repair of certain types of DNA damage or that help cells to survive the damage. The current chapter relates the stories of the first two on the list (xeroderma pigmentosum and Cockayne syndrome).

Table 22.1.

Genetic disease	Defective process	Defective genes	Chapter	
Xeroderma pigmentosum (XP)	Nucleotide excision repair	*XPA-XPG; XPV*	22, 23	
Cockayne syndrome	Transcription-coupled repair	*CSA, CSB*	22	
Fanconi anemia	Inter-strand crosslink repair	FANC genes	31	
Ataxia telangiectasia	Cell cycle checkpoint activation	*ATM, ATR*	29	
Lynch syndrome	DNA mismatch repair	*MLH, MSH, PMS*	25	
Prone to breast cancer	Homologous recombination	*BRCA1, BRCA2*	26	
Li-Fraumeni syndrome	"Guardian of the genome"	*TP53*	32	
Bloom's syndrome	Premature aging in childhood	*BLM*		
Werner's syndrome	Premature aging in adult	*WRN*		

The Xeroderma pigmentosum (XP) story: a curse and a clue.

The esoteric name, xeroderma pigmentosum (or simply XP), became common parlance among researchers and clinicians dealing with skin diseases and cancer. Likewise, the name will appear so many times in the chapter that the reader may find it enter his/her familiar vocabulary. Literally, xeroderma pigmentosum means "pigmented dry skin," but that benign-sounding name does not match the severity of the disease. Children affected with this fortunately very rare inherited disease came to be called children of the dark and even vampire children, because for them daylight could be deadly. If they failed to adhere to a discipline of the dark, many of them had little chance of surviving beyond the age of 20. However, some who practiced sun protection led nearly normal lives. Their quandary of how to meld their extreme light sensitivity with their social lives became the subject of several films ("Children of the Dark" – TV movie 1994). It may not have consoled these affected people very much to know that their suffering would lead to new knowledge that would help others, including many suffering with cancer.

Children with xeroderma pigmentosum (XP) tended to get freckle-like pigmentation in sun-exposed skin, severe burns after minimal sun exposure, and damage to sun-exposed parts of the eyes with loss of vision and ocular cancer. At least 8 molecular defects were discovered. About 25% of the patients had progressive neurological degeneration. Their greatest risk of sun exposure, however, was potentially deadly skin cancers.

The older medical literature often showed patients with advanced stages of the disease. Since it affects sun-exposed skin, its effects are exposed to view. The worst of it, however, was hidden: some of the skin nodules become malignant cancers that spread to organs within the body. Xeroderma pigmentosum was one of the most cancer-prone conditions with a 10,000-fold increase in skin cancer (Bradford et al., 2011). It became one of the most extensively studied diseases in all of cancer research. The advanced cases commonly shown in the medical literature look so bad, that I have selected an early and relatively mild case to show in Figure 22.1 (Anderson, 1889).

The disease was first described by Ferdinand Ritter von Hebra and Morris Kaposi in 1874, who coined the name "xeroderma pigmentosum" (*Ueber Xeroderma pigmentosum. Medizinische Jahrbücher*, Wien, 1882: 619–633). The case in Figures 22.1 and 22.2 dates back to 1889, when its inherited nature was known, but its cause was still clouded in mystery (Anderson, 1889). Up to that time, only 44 cases of the disease were known. This case was reported by T. McCall Anderson of the University of Glasgow in the British Medical Journal. It was of a 9-year old boy, who, despite his skin lesions and the loss of his left eye -- which was removed because it had a tumor growing on it -- still looked to be otherwise in generally good health. You can see from Figure 22.1 that his skin lesions were mainly on the face, neck, upper shoulders, and lower arms: the parts of his body that would have been most exposed to the sun. Microscopic examination of his tumors was noted to be typical of epithelioma (epithelial cancer) (Figure 22.2). His parents were both in good health, but his only sister was similarly affected and had died at the age of 9. This was consistent with the known recessive inheritance of the disease. The exact genetics however were confusing; we now know the reason for the confusion: it was because the disease,

with clinical variation, can be caused by mutation of any one of several genes, located on different chromosomes (Table 22.2).

Figure 22.1. Woodcut of a 9-year old boy with xeroderma pigmentosum (Anderson, 1889). The child had multiple cancers on his face, and his left eye was removed because there was a cancer in it. Unless protected against sunlight his condition could have become much worse. At the time this picture was made (circa 1889), little was known about the disease. Pigmentation (darkening) is also shown in the sun-exposed areas around his neck and the lower parts of his arms.

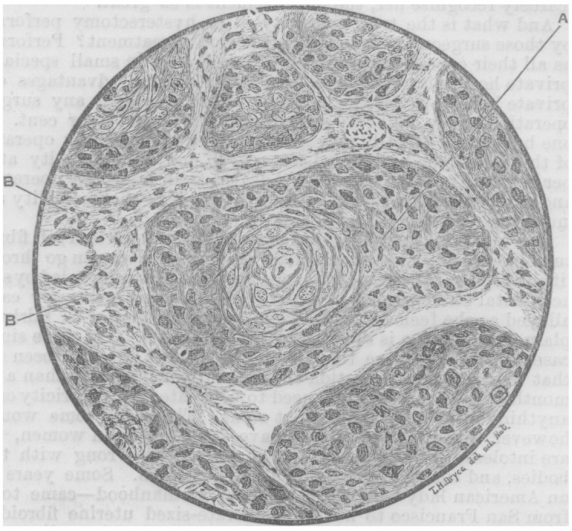

Figure 22.2. A micrograph (x250) of a skin tumor from the patient in Figure 22.1 (Anderson, 1889). The central portion (labeled A in the micrograph) was typical for a squamous carcinoma where the cancer retained a bizarre memory of the arrangement of the multilayered cells of the outer skin. The cancer cells showed the usual large irregular nuclei, compared with the fibroblasts in the stroma (labeled B) within which the tumors are growing. A blood vessel is seen on the left.

Another case of xeroderma pigmentosum, also published in 1889 in the British Medical Journal, was of a 10-year-old boy. The report noted the role of sun exposure by the unusual involvement of this child's feet and lower legs (Hunter, 1889):

> *It may be remarked here that these children went to school during the summer months without shoes or stockings and the trousers often rolled up. The latter is done so as not to interfere with running or jumping, and in wet weather to keep them (the trousers) clean. This is a common habit in the country districts, and thus, in these cases, the feet and legs which are affected were also exposed* (Hunter, 1889).

These two reports (Anderson, 1889; Hunter, 1889) gave a detailed description of the skin lesions and the course of the disease in all-together 4 patients. These early reports, while little was as yet known about the disease, show what physicians of ~130 years ago saw and experienced.

Next, we move ahead 78 years. Several different forms of the disease were by then known with different clinical manifestation, although all of them entailed skin lesion caused by sunlight. The cells from patients having mutations in a particular gene were referred to as a "complementation group" because the normal version of the respective gene could cure the defect in the corresponding mutant cells. The defect measured was "unscheduled DNA synthesis" which will be explained in a moment. A complementation group, in common parlance, became nearly the same as referring to a particular mutant gene (Table 22.2).

It was 1967, I think, in a small conference in the NIH Clinical Center; we had a guest speaker. It was James E. Cleaver from the University of California. He claimed to have detected a defect in unscheduled DNA synthesis in cells from patients with xeroderma pigmentosum. Some of us were skeptical, because we had expected that phenomenon, but no one had yet been able to demonstrate it. Moreover, his data seemed to be barely above the noise level. Within a year or so, however, he published more convincing data (Cleaver, 1968) and the skeptics were soon on the bandwagon as their investigations corroborated and extended Cleaver's claims (Kraemer and DiGiovanna, 2015).

So now, what is "unscheduled DNA synthesis" and how is it related to DNA repair? Well, our concept was that cells whose DNA was damaged by ultraviolet light (UV) would be undergoing a small amount of DNA synthesis for repair after the damage was cut out. This would be occurring even in cells that were resting, i.e., not cycling in cell division and hence not undergoing the normal DNA synthesis phase. Normally, those cells would not be synthesizing DNA. But to repair DNA damage, even those cells would be undergoing a small amount of unscheduled DNA synthesis: it was "unscheduled" because it would be occurring even if the cells are not in the cell division cycle. We had reason to believe that cells from xeroderma pigmentosum (XP) cells were defective in their ability to repair DNA damage caused by ultraviolet light. Therefore, they might not exhibit unscheduled DNA synthesis, as Cleaver had shown to be the case, namely the absence of unscheduled DNA synthesis in UV-irradiated XP cells.

To put it in another way: exposing normal skin to ultraviolet light induced DNA damage, which was promptly repaired by a process that involved a small amount of DNA synthesis to replace the damaged DNA regions that were cut out by the repair mechanism. This DNA synthesis would be occurring even in cells that were not in division cycle and hence would not ordinarily undergo any DNA synthesis. Since that DNA repair synthesis would be occurring in quiescent (*i.e.* non-dividing, non-cycling) cells, it was termed "unscheduled." Xeroderma pigmentosum patients were presumed to be defective in such DNA "excision" repair, and their cells therefore would be unable to carry out unscheduled DNA synthesis.

The inability of skin cells from xeroderma pigmentosum (XP) patients to exhibit unscheduled DNA synthesis in response to ultraviolet light (UV) was later confirmed directly in patients (Figure 22.4) (Epstein et al., 1970). A small region of the patient's skin was irradiated with ultraviolet light; then, tritium-labeled thymidine was injected under the irradiated portion of skin, which was then biopsied and radiographed to show the cells that had incorporated the radioactive thymidine into DNA (Figure 22.4). I do not know whether the patients had given informed consent for this procedure of injecting radioactive material into the skin – it would probably not be permitted now with or without informed consent.

As thymine dimers were being cut out from DNA of UV-irradiated cells, transient DNA strand breaks occurred. Detection and study of that step became possible using the DNA filter elution technique described in Chapter 9. Before that, the available methods were not sensitive enough to detect those breaks. Application of the filter technique allowed Al Fornace, who had recently joined my lab as a post-doctoral fellow, to fill in that gap in the evidence (Fornace et al., 1976). We showed, as expected, that DNA strand breaks appeared after UV exposure of normal cells, but not in XP cells (Figure 22.5).

Using the filter methods, Fornace and I also showed that UV produces DNA-protein crosslinks and that XP cells were unable to repair them (Fornace and Kohn, 1976) and later confirmed the inability of XP cells to repair DNA-protein crosslinks by using *trans*platin to produce them (Chapters 3 and 9) (Fornace and Seres, 1982). Moreover, the XP cells had increased vulnerability to being killed by *trans*platin. Those results showed that the nucleotide excision repair (NER) pathway was able to recognize and repair, not only thymine dimers, but also DNA-protein crosslinks.

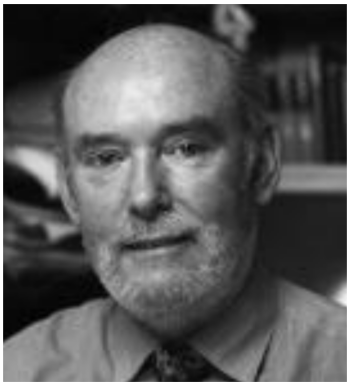

Figure 22.3. James Edward Cleaver (1938-), discoverer of the molecular process that is defective in the genetic disease, xeroderma pigmentosum. This was the seminal discovery of a human DNA repair deficient disease.

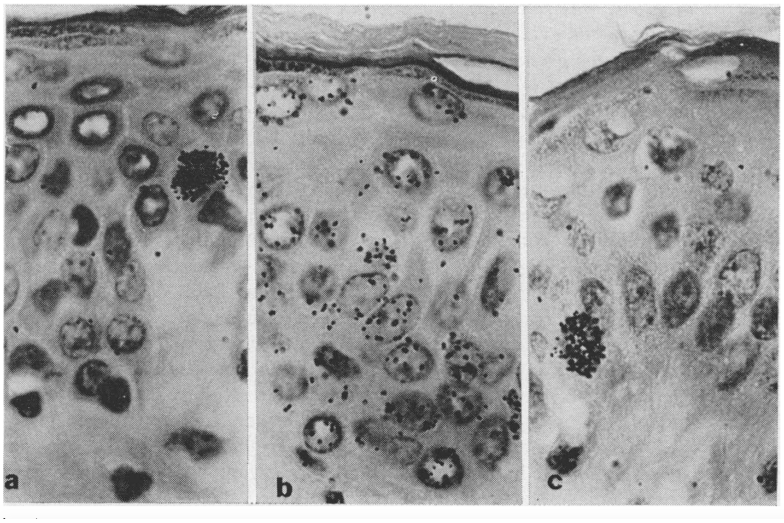

Figure 22.4. Skin from a xeroderma pigmentosum patient was unable to repair DNA damage caused by ultraviolet light. The repair entailed a small amount of DNA synthesis to replace the damaged DNA regions. These studies demonstrated defective DNA repair synthesis ("unscheduled DNA synthesis") in skin of xeroderma pigmentosum patients. *Left*, normal skin: no radioactivity grains. *Middle*, irradiated normal skin: many cells show radioactivity grains, indicative of unscheduled DNA synthesis. *Right*, irradiated xeroderma skin: no unscheduled DNA synthesis (the heavily labelled cell is undergoing replicative DNA synthesis) (Epstein et al., 1970).

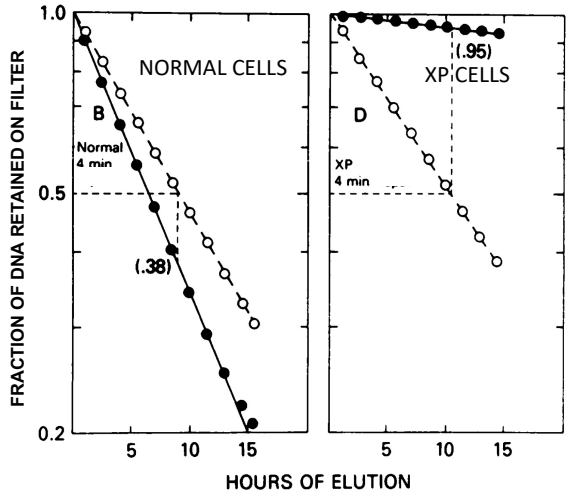

Figure 22.5. The cutting out of UV-induced thymine dimers in the course of nucleotide excision repair (NER) was expected to produce transient DNA strand breaks, but it had been difficult to detect these breaks. The DNA filter elution methods described in Chapter 9, however, were sensitive enough to show up those transient breaks – shown by a high DNA elution rate (solid circles in the *left* panel). Cells from a xeroderma pigmentosum (XP) patient showed almost no DNA elution (sold circles in the *right* panel), as expected when thymine dimer excision is defective (Fornace et al., 1976). (The open circles were for internal standards used to increase the precision of the measurements.)

An unusual variant of XP is discovered.

As often happens in science, a new discovery, when further investigated, becomes challenged by findings that don't fit the original concept. And so it was that the fourth NIH XP patient had the high light sensitivity of the skin, but showed no defect in unscheduled DNA synthesis (Robbins et al., 1974). About the same time, Jim Cleaver reported 3 more XP patients whose cells had normal unscheduled DNA synthesis after being exposed to ultraviolet light (Cleaver, 1972). Those patients defined a variant of XP that had normal nucleotide excision repair (NER); this variant of XP became known as XPV. The defect in XPV turned out to be a mutation in a special DNA polymerase, known as Pol-eta or PolH, which is needed to synthesize DNA across a defect in the template strand, such as may remain after removal of pyrimidine dimers (DiGiovanna and Kraemer, 2012). Cleaver later remarked that, if his first patient had been of the XPV type, he might have erroneously concluded that XP had normal DNA repair (Kraemer and DiGiovanna, 2015).

Subtypes of xeroderma pigmentosum: complementation groups

Even among XP patients whose cells were defective in unscheduled DNA synthesis, there was considerable variability in how severe the disease was, how sensitive the cells were to be killed by ultraviolet light, and the clinical picture in general. Particularly puzzling was that the disease of some patients had neurological symptoms, sometimes quite severe.

An extreme case of the latter was a syndrome that deserved its own name: De Sanctis–Cacchione syndrome. It was one of the rarest, most severe forms of xeroderma pigmentosum (XP). In addition to being highly sensitive to sunlight, these XP patients were of short stature and developed progressive neurologic degeneration. The syndrome was first recognized by de Sanctis and Cacchione in 1932, who described three brothers with XP who had microcephaly, mental deficiency, dwarfism, gonadal hypoplasia, progressive neurologic deterioration, deafness, and ataxia beginning at the age of 2 years. This new syndrome was described in 1932 in an obscure Italian journal: de Sanctis C., Cacchione A. *L'idiozia xerodermica. Riv Sper Freniatr Med Leg Alien Ment.* 1932;56:269–292.

The cause of these different types of XP could only be investigated after new methods and concepts were developed, which took several decades. The story began to unfold in 1971 when Dirk Bootsma (Figure 22.6), a Professor of Genetics at Erasmus University in Rotterdam, thought that the large differences in the clinical picture of XP patients might be due to different genes, each of which, when mutated, would cause a particular clinical form of the disease. He reasoned that, if two of those defective genes, each on a different chromosome, were put into the same cell, they might complement each other and restore normal unscheduled DNA synthesis. He wanted to test that idea using cells from two very different forms of XP: the classic variety and the De Sanctis–Cacchione type.

But how could one put the presumed different defective genes into the same cell? Bootsma and his colleagues developed a method that used the ability of certain viruses (inactivated Sendai virus) to fuse cells together to produce cells that sometimes had two nuclei. However, although some of the binucleate cells had a nucleus from each of the XP types, oftentimes they had nuclei from the same type. In order to distinguish whose different cases, they used a clever trick: they fused cells from a male patient who had one XP type with cells from a female patient who had the other XP type of the disease. They then exposed the cells to UV to produce DNA damage. By carefully examining the chromatin in each nucleus of a binucleate cell, they could tell whether the nucleus came from a male or female cell. They indeed found that those and only those binucleate cells that had both a male and a female nucleus – one nucleus from each XP type -- complemented each other to restore unscheduled DNA synthesis (Figure 22.7) (De Weerd-Kastelein et al., 1972).

Other investigators, particularly Ken Kraemer at NIH (Figure 22.8), then jumped in and, using a modified method, found that there were in fact several different complementation groups among the XP patients, their cells, and their mutated genes (Table 22.2). Decades later, the genes that were mutated in the various complementation groups were cloned and their functions determined. Quite remarkably, the proteins produced by all of these XP genes were found to work together to repair DNA damage by a very important mechanism: DNA nucleotide excision repair (NER). How this mechanism works is the subject of the next chapter. How complementation groups were determined is explained in Figure 22.9.

Figure 22.6. Dirk Bootsma (1936-), a Professor of Genetics at Erasmus University Rotterdam, Netherlands, developed a cell-fusion method by which he discovered xeroderma pigmentosum complementation groups. (From *Ned Tijdschr Geneeskd* 2002 12 oktober;146(41).)

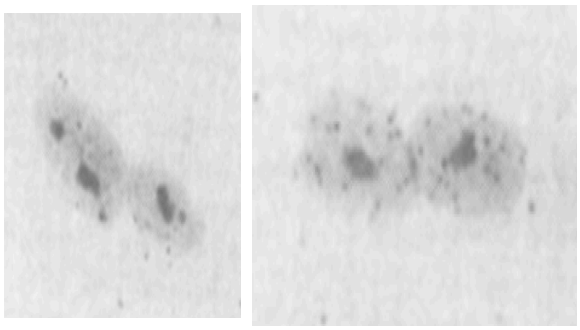

Figure 22.7. How Bootsma and his colleagues showed that a gene responsible for what was then called the classic type of XP and a gene responsible for the De Sanctis–Cacchione type of XP complemented each other to restore the unscheduled DNA synthesis process of DNA repair (De Weerd-Kastelein et al., 1972). They fused cells from a male child who had one type of XP with cells from a female child who had the other type of XP. Each panel shows a cell with two nuclei. The cells had been exposed to UV to produce DNA damage and then incubated with radioactive thymidine. The cell on the right shows radioactive spots scattered in both nuclei where unscheduled DNA synthesis was occurring. The cell on the left shows no unscheduled DNA synthesis. The nuclei in the cell on the left both came from male donors. The same was true if both nuclei came from females. Only cells that had one male and on female nucleus – therefore, a mixture of the XP types – showed unscheduled DNA synthesis: the cell had a good copy of both genes, one from each nucleus.

Figure 22.8. Kenneth Kraemer (*left*) and Vilhelm Bohr (*right*) in 2005, commemorating four decades of research on DNA repair at NIH. Kraemer received an MD degree at Tufts Medical School and became board certified in Dermatology and Internal Medicine. He came to NIH in 1971 as a clinical associate in the Dermatology Branch and has been leading ground-breaking research on xeroderma pigmentosum and related diseases. In 1980, he established and since co-chaired with Vilhelm Bohr an NIH Special Interest Group on DNA Repair in which he pioneered internet

conferencing to bring together researchers from several institutions in different cities. Bohr, a descendent of Niels Bohr, received an MD degree in 1978, followed by PhD and D.Sc. degrees in 1987 from the University of Copenhagen in Denmark. Together with Philip Hanawalt at Stanford University, he pioneered investigations of transcription-coupled DNA repair, which he continued in my Laboratory. In 1992, he became Chief of the Laboratory of Molecular Genetics in the National Institute of Aging where he has been leading studies of DNA repair and cancer.

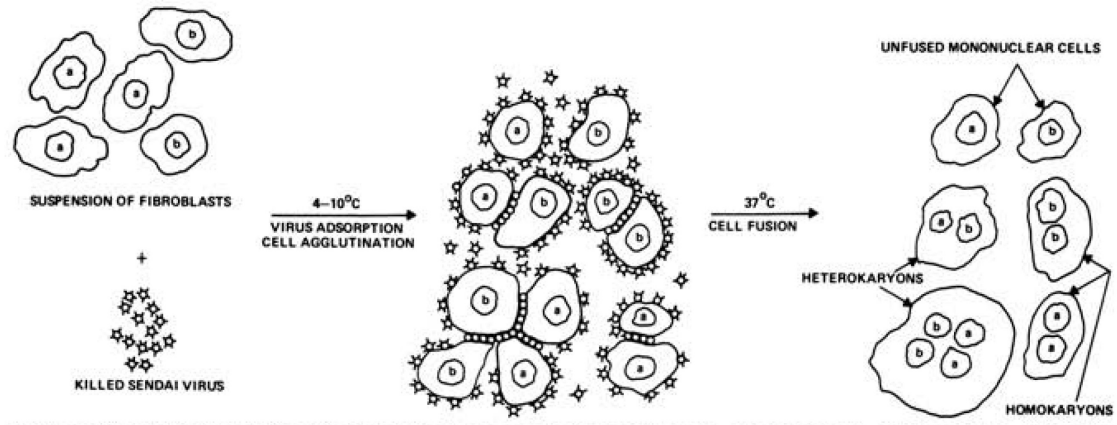

Figure 22.9. Complementation was determined by fusing together a mixture of cells from two different xeroderma pigmentosum (XP) patients. Complementation occurred when the patients had defects in different XP genes: combining their cells would give at least some fused cells that did not show the defect – because some of the fused cells would have a good copy of both genes. The patients therefore were of two different complementation groups. On the other hand, if the cells from the two patients had defects in the same in the same gene, then all of their fused cells continued to show the defect: the two patients were of the same complementation group. The experiments utilized killed Sendai virus, which caused the cells to stick to each other. When the cells were then warmed up, the cells fused together and many of them had two nuclei, showing that they were a combination of two different cells. If some of the binucleate cells restored the defect present in both of the original cell strains, then complementation was said have occurred and that the cells came from different complementation groups. If none of the binucleate cells came from cells that all had defects in the same gene, then they were of the same complementation group. (From (Robbins et al., 1974).)

Table 22.2. Xeroderma pigmentosum genes (complementation groups)

Complementation Group	Genes / proteins / definitions	Chromosome
XP-A	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.)

The Cockayne syndrome story

But the XP story had yet another twist in a different, but closely related, genetic disease. In 1936, Edward Alfred Cockayne, a pediatrician at the Great Ormond Street Hospital for Sick Children (which still stands (Figure 22.10)) in Bloomsbury, London, described two children with a previously unknown syndrome that was to bear his name (Figure 22.11) (Cockayne, 1936). He characterized the syndrome as "dwarfism with retinal atrophy and deafness." Although Cockayne's syndrome became closely linked with xeroderma pigmentosum, and these children had sun sensitivity, their skin was clear (Figure 22.11). That 85-year-old paper was not easy to find and may become increasingly difficult to find as time goes by; therefore, I am reproducing here Cockayne's original description of the syndrome, as well as images he included in his paper (Figures 22.12-14):

The two children with this dystrophy, a girl aged seven years and eleven months and a boy aged six years and three months were admitted to the Hospital for Sick Children, Great Ormond Street in June, 1935. The parents, who are natives of north Hampshire, are of English race, normal and not blood-relations, and they have been unable to trace the occurrence of the condition in their ascendants or collaterals . . .

. . .

The dwarfs are so much alike in facial appearance, build and disposition, that the same general description will suffice. Both have small heads, that of the girl being the smaller, but, although the vault of the skull is flattened and the circumference small, the general shape is normal, and neither child has the receding forehead characteristic of microcephaly. Their faces are small with sunken eyes and prominent superior maxillae. They are slightly built with short, slender trunks and unduly long legs, and their feet and hands are too large in proportion. The third and fourth fingers of their hands are deviated a little towards the mesial line. Both are active and their movements are quick and bird-like. They are friendly and playful, invariably good tempered, and laugh with obvious enjoyment at the slightest provocation. Although they are imitative, they have a certain amount of initiative and in playing with toys are no more destructive than most children of their age and class. They frequently make noises

which at first sound like speech, but actual words can seldom be recognized, although the girl has been heard to say ' mother ' and ' do it again ' and the boy has said ' doctor ' several times. They do not answer to their names or obey spoken words, nor do they take any notice of a sound made behind their heads, but they are quick to obey signs. Mr. James Crooks, F.R.C.S., who saw them, says that although not totally deaf, their hearing is greatly impaired. It is difficult to tell how much of their backwardness is due to deafness and how much to mental deficiency. Their behaviour is not the usual behaviour of deaf children. They appear to be a little below the average in intelligence and are far more excitable and laugh much more readily than children of normal mentality whether deaf or not.

Children with Cockayne syndrome rarely survived to adulthood.

Figure 22.10. The Great Ormond Street Hospital for Sick Children in Bloomsbury, London, where Edward Alfred Cockayne saw two children in 1935 who had a new syndrome, which came to bear his name (Cockayne, 1936). (Creative Commons, Wikipedia)

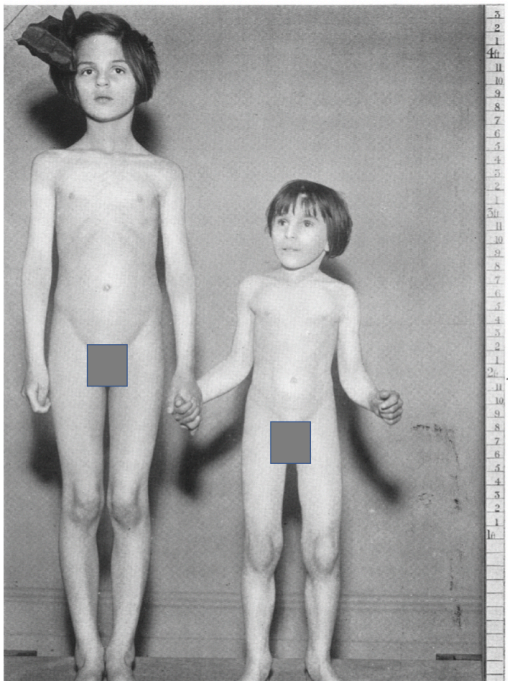

Figure 22.11. A 7-year old girl with the syndrome described by Cockayne in 1936 (*right*) standing next to a normal girl of the same age (left) (Cockayne, 1936). The affected child, although much shorter than normal, had relatively long legs and large hands. Her head, however, was relatively small. Her skin was clear and had no sign of the sun-induced damage that is characteristic in xeroderma pigmentosum.

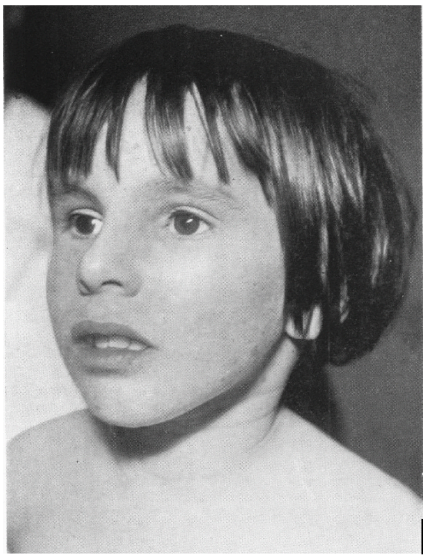

Figure 22.12. Cockayne said the affected child had an abnormally small head with sunken eyes and prominent front upper jaw (Cockayne, 1936).

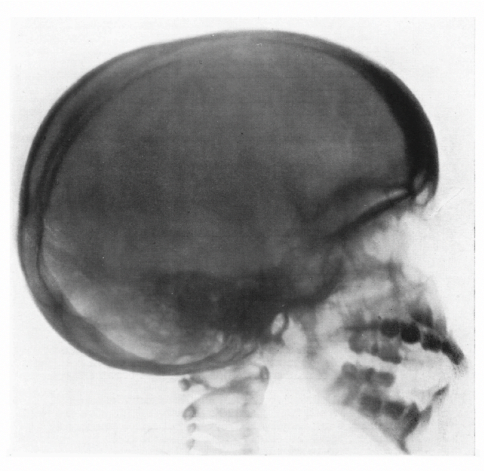

Figure 22.13. Cockayne described her skull as being small of small circumference with thickened bones and prominent upper jaw (Cockayne, 1936).

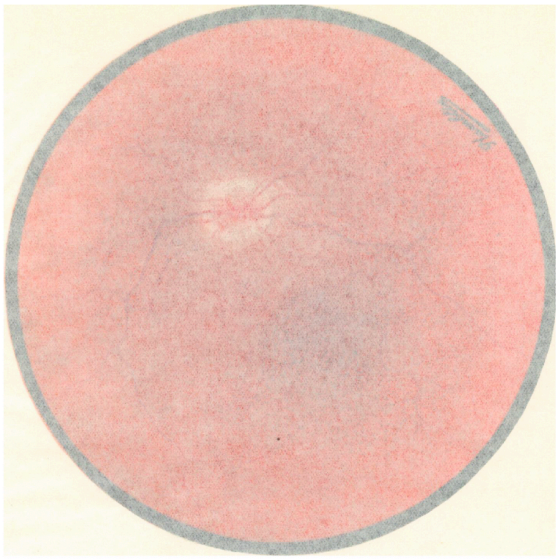

Figure 22.14. The retinal atrophy Cockayne noted in the affected children (Cockayne, 1936). He noted markedly narrowed retinal arteries and atrophic changes, particularly in the central region of the retina.

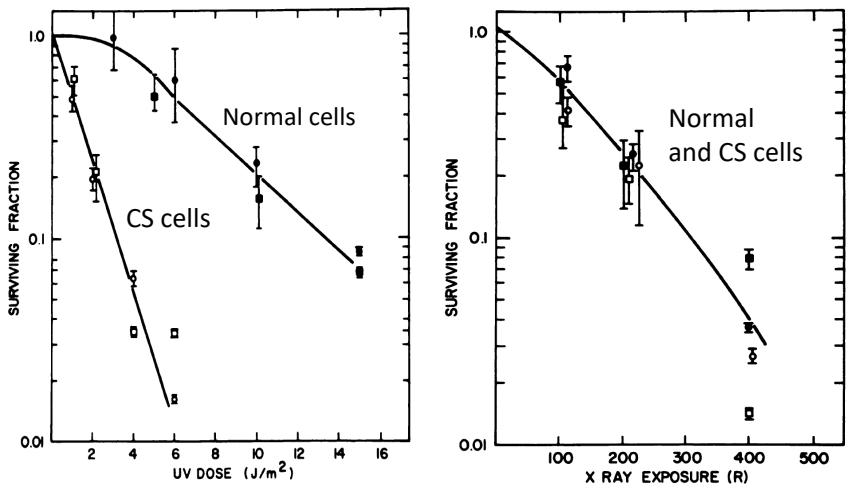

Figure 22.15. Fibroblast cells from the skin of Cockayne syndrome (CS) children were unusually sensitive to being killed by ultraviolet light (UV) (*left*) but had normal sensitivity to x-rays (*right*) (Schmickel et al., 1977). Xeroderma pigmentosum cells had the same pattern: high sensitivity to UV but not to x-rays.

It was four decades after Cockayne's description before the first clue to the cause of the syndrome arrived. It came from the laboratory of Schmickel and coworkers at the University of Michigan. They showed that fibroblast cells derived from the skin of affected children were unusually sensitive to ultraviolet light (UV), whereas their sensitivity to x-rays was normal (Schmickel et al., 1977) (Figure 22.15). This curious difference of being highly sensitive to DNA damage caused by UV but not to DNA damage caused by x-ray was exactly the same as in xeroderma pigmentosum (XP). However, despite the cells of most Cockayne's syndrome (CS) children being highly sensitive to UV, the children were not highly sensitive to sunlight *and* their cells removed thymine dimers from their DNA at a near normal rate, contrary to the inability of xeroderma pigmentosum cells to remove those dimers (Schmickel et al., 1977). It took almost another decade to resolve the puzzle of why CS cells resembled XP cells in being unusually sensitive to UV, even though they (the CS cells) removed thymine dimers from their DNA normally, as opposed to the inability of XP cells to do so.

The confusing findings about the relationship between Cockayne syndrome and xeroderma pigmentosum began at last to be clarified by Vilhelm Bohr (Figure 22.8), working at Stanford with Philip Hanawalt and later at NCI in my Laboratory. He pinned down a special nucleotide excision repair (NER) mechanism designed specifically and exclusively to repair DNA damage in regions of the genome that were being actively transcribed at the time that the damage was present. It was a distinct type of repair and was named "transcription-coupled nucleotide excision repair" (TCNER) (Bohr et al., 1985). Researchers in The Netherlands and the UK then showed that Cockayne's syndrome had a defect in TCNER (Venema et al., 1990).

But why did a defect in TCNER (due to a defective CS gene) make cells sensitive to being killed by UV (Figure 22.15)? The reason was that transcription (RNA synthesis), as it

progressed along the DNA, occasionally collided with UV-induced thymine dimer (or other pyrimidine dimer). The collision produced a peculiar DNA damage configuration that was apt to lead to death of the cell. This disaster was avoided by a special NER machinery (TCNER) that was attached to transcription machinery. When transcription encountered a UV-induced dimer, it was promptly excised by TCNER, allowing the transcription machinery to continue merely on its way. The TCNER machinery in Cockayne syndrome (CS) cells however was defective, which put the cells at risk whenever transcription collided with a UV-induced dimer. Thus, while most of the dimers scattered in the genome were efficiently removed by NER, the small fraction of dimers involved in transcription-collision needed the special TCNER to be removed. Consequently, CS cells were killed by UV even though the large majority of dimers were removed from their DNA.

However, it remained puzzling why different cases of Cockayne's syndrome sometimes had different patterns and severities of the symptoms, and there were cases that had both Cockayne syndrome and xeroderma pigmentosum symptoms (Nance and Berry, 1992). As so often happens in research, the real world, as opposed to simpler worlds of theory, hides complications that challenge researchers, as in a treasure hunt. More recent findings, it turns out, indicate that the molecular defects in Cockayne's syndrome have more widespread aspects, including defects in base excision DNA repair and mitochondrial functions (Karikkineth et al., 2017).

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