Nucleotide Excision Repair

International Edition: DOI: 10.1002/anie.201601524
German Edition: DOI: 10.1002/ange.201601524

Mechanisms of DNA Repair by Photolyase and Excision Nuclease (Nobel Lecture)**

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circadian clock · cryptochrome · Maxicell method · nucleotide excision repair · photoreactivation

Introduction

The ultraviolet (UV) wavelengths in sunlight damage DNA by converting two adjacent thymines into a thymine dimer (T<>T) which is potentially mutagenic, carcinogenic, or lethal to the organism. This damage is repaired by photolyase in *E. coli* and by the nucleotide excision repair system in *E. coli* and in humans. In this lecture I will present our work on photolyase and nucleotide excision repair, and I will conclude my talk by describing how our research on photolyase led to the discovery of an essential circadian clock protein, called cryptochrome, that links these two research subjects to one another and thus completes the circle.

Photolyase

Photolyase is a photon-powered nanomachine that uses blue light photons to repair thymine dimers that are induced in DNA by UV. Photolyase was discovered by my PhD mentor Claud S. (Stan) Rupert (Figure 1) in 1958, and this discovery marked the beginning of the field of DNA repair as a scientific discipline. Decades before the discovery of photolyase, it had been known that UV kills bacteria very efficiently (Figure 1, right panel). In 1949, Kelner, from Cold Spring Harbor, made the interesting observation that if bacteria killed by UV were exposed to visible light, the dead bacteria were miraculously brought back to life.[1,2] However, he had no explanation for this phenomenon, which was termed photoreactivation. Rupert analyzed this phenomenon further. He demonstrated that UV killed bacteria by damaging their DNA, and that there is an enzyme (photoreactivating enzyme = photolyase) that uses the blue light energy in visible light to repair DNA damage. Blue light, thus, brings dead cells back to life, demonstrating that this resurrection from the dead was not a miraculous phenomenon that needed a metaphysical explanation,[3,4] but could instead be explained by the laws of physics. The reaction mechanism that Dr. Rupert developed is as follows:[5–7] UV converts two adjacent pyrimidines, including thymines, to a CPD (cyclobutane pyrimidine dimer), and there is an enzyme called photolyase that uses blue light energy to break the two abnormal bonds joining the thymines and thus converts the thymine dimer to two normal thymines (Figure 1, bottom panel). Photolyase therefore repairs DNA and eliminates the harmful effects of UV. While this was a satisfactory explanation of the photoreactivation phenomenon, it raised a physical question: Photolyase is a protein, and proteins do not absorb blue light. Therefore, for the next two decades Rupert and many other researchers attempted to identify the blue light-absorbing component of photolyase. They were unsuccessful because Rupert had determined that an *E. coli* cell contains

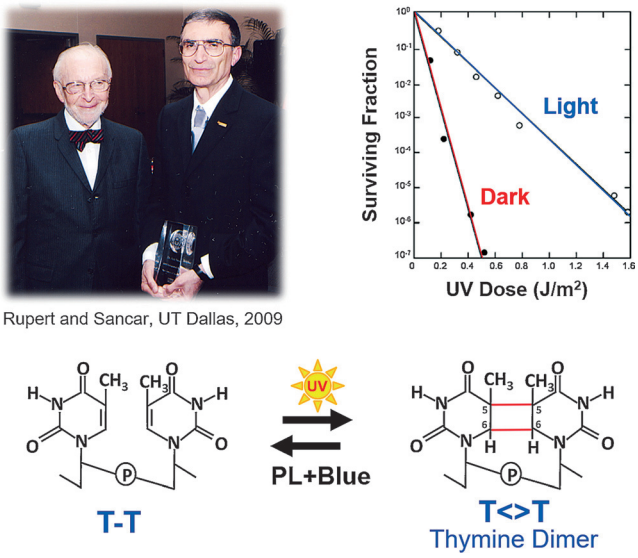

Figure 1. Photoreactivation and photolyase. Top left: Claud S. Rupert and Aziz Sancar at a function at the University of Texas at Dallas in 2009. Top right: Photoreactivation in *E. coli*. An *E. coli* strain defective in nucleotide excision and recombination repair and carrying the cloned photolyase gene was irradiated with the indicated UV dose and either plated directly (closed circles) or plated after exposure to a camera flash of 1 millisecond (open circles). Reproduced from Ref. [9]. Bottom: General model for photolyase based on Rupert's pioneering work: UV induces the formation of a cyclobutane thymine dimer (T<>T), photolyase binds to the dimer, absorbs a blue light photon, and converts the dimer to two canonical thymines.

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only 10–20 molecules of photolyase, and this made it virtually impossible to purify and characterize the enzyme.

In 1974 when I joined Dr. Rupert's lab, gene cloning had just been developed at Stanford University. As a fresh graduate student, I thought I could do anything I wanted, and therefore I proposed to Dr. Rupert to clone the photolyase gene, overproduce the enzyme, and purify it. He said, "Go ahead." After months of work, I successfully cloned the gene.[8,9] An electron micrograph of the plasmid containing the photolyase gene is shown in Figure 2 (left). In subsequent

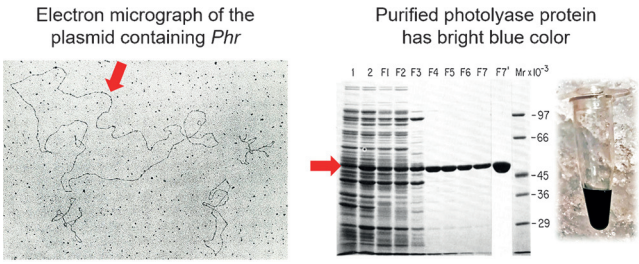


Figure 2. Cloning and purification of photolyase. Left: Electron micrograph of the plasmid carrying the photolyase gene (*Phr*). Reproduced from Ref. [8]. Middle: Purification of photolyase from an *E. coli* strain overproducing the protein. Reproduced from Ref. [10,11]. Right: Purified photolyase exhibits a blue color because of the flavin neutral radical cofactor of the enzyme. The color ranges from sky blue to dark blue depending on the concentration of the enzyme.

years at the University of North Carolina, my colleagues and I used the cloned gene to purify the enzyme in gram quantities (Figure 2, middle),[10–12] and while purifying it we found that it has a bright blue color (Figure 2, right).[12] That finding, without any chemical analysis, answered the physical question: It has a blue color which means it absorbs light. We proceeded to identify the light absorbing component of the enzyme using analytical chemistry, and to our surprise, we found that it contained not one, but two blue light-absorbing cofactors, which are methenyltetrahydrofolate (folate) and two-electron reduced and deprotonated flavin adenine dinucleotide ($FADH^-$).[13–28] Moreover, we found that the enzyme exhibits colors ranging from purple to orange depending on the redox state of the flavin cofactor[29] (Figure 3). We next determined the functions of the two cofactors by carrying out photochemical experiments. We found that the folate acts like a solar panel, absorbing light and transferring the excitation energy to $FADH^-$.[15–17,24] The flavin is the actual catalyst, and upon excitation by energy transfer from folate (and less efficiently by direct absorption of a photon) it carries out the repair reaction on the CPD by a radical mechanism through a cyclic redox reaction.[24,26] To provide structural basis for the proposed reaction mechanism, we collaborated with Johann Deisenhofer to crystallize photolyase and obtain the 3D structure of the enzyme,[30] which is shown in Figure 4 in ribbon diagram and surface charge representations. As predicted from the biochemical experiments, the folate is like a solar panel, where it sits on the "roof" of the enzyme, absorbs light, and then transfers the light energy to the flavin cofactor within the core the enzyme so to carry out catalysis.

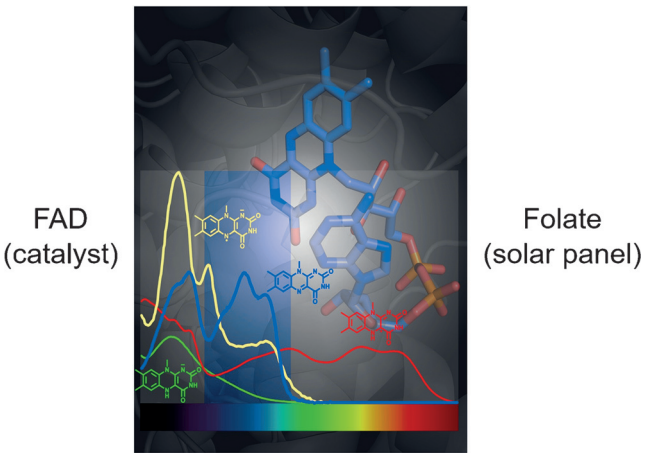


Figure 3. Photolyase chromophores. Photolyase from *E. coli* contains two chromophores, which are two-electron reduced flavin adenine dinucleotide ($FADH^-$) and methenyltetrahydrofolate (folate). The folate is the solar panel (or photoantenna) and the flavin is the catalytic cofactor. During purification, the flavin undergoes changes in oxidation state and as a consequence the enzyme may exhibit colors ranging from purple to orange. The figure shows the 4 redox states of flavin, and the corresponding absorption spectra, superimposed on the crystal structure of the active site of the enzyme. Image courtesy of Dongping Zhong.[29]

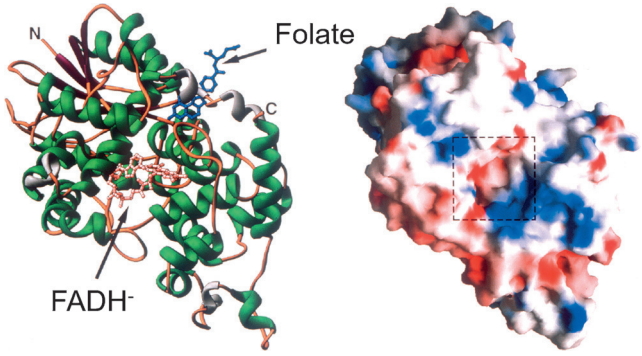


Figure 4. Crystal structure of *E. coli* photolyase. Left: Ribbon diagram representation. Right: surface potential representation. Positively and negatively charged residues are highlighted in blue and red, respectively. The phosphodiester backbone of the damaged strand binds to the positively charged diagonal groove on the enzyme surface. The dashed box marks the hole leading to the $FADH^-$ catalytic cofactor. Reproduced from Ref. [30].

With this general structural view, then, the mechanism of photolyase was developed (Figure 5):[31] Photolyase binds DNA containing a CPD because the T<>T distorts the backbone of the DNA. Upon binding to damaged DNA, through ionic interactions between the positively charged groove on the photolyase surface and negatively charged DNA phosphodiester backbone the enzyme pulls the T<>T out from within the helix and into the core of the enzyme so that the T<>T is within Van der Waals contact with $FADH^-$. It makes a very staple complex, and nothing happens until folate absorbs a photon and transfers the excitation energy to the flavin cofactor. The excited-state flavin, $FADH^{-}$*, repairs

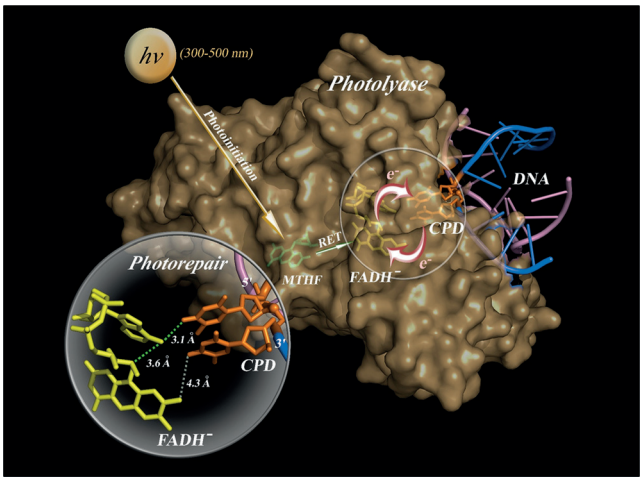


Figure 5. Reaction mechanism of photolyase. The enzyme makes ionic bonds with the phosphate residues of the damaged DNA strand and flips out the thymine dimer dinucleotide into the active site cavity so that the T<>T is within Van der Waals contact with $FADH^-$. The catalytic reaction is initiated by absorption of a photon (300–500 nm) by the folate (MTHF). The MTHF excited singlet state transfers the excitation energy to $FADH^-$ by Förster resonance energy transfer (FRET). The excited $FADH^-$* splits the cyclobutane ring by cyclic redox reaction to convert T<>T to T–T, and the repaired DNA dissociates from the enzyme. The inset shows the distances between the indicated atoms of $FADH^-$ and the cyclobutane pyrimidine dimer (CPD). Image courtesy of Dongping Zhong.[29,33]

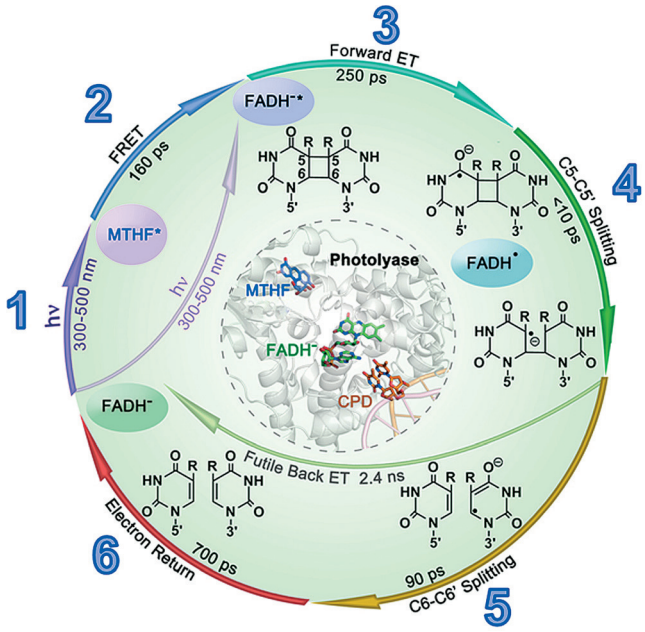


Figure 6. Microscopic rate constants for photolyase. The rate constants were determined by ultrafast time-resolved absorption and fluorescence up-conversion spectroscopy. The cleavage of the cylclobutane ring is by a concerted asynchronous mechanism which couples the cleavage of the C5—C5 bond in less than 10 ps to cleavage of the C6—C6 bond in 90 ps. The entire photochemical reaction is complete in 1.2 ns, with an overall quantum yield of about 0.9. The inner circle shows the relative locations of the photoantenna (MTHF), the catalyst ($FADH^-$) and the thymine dimer substrate (CPD). Image courtesy of Dongping Zhong.[29,33]

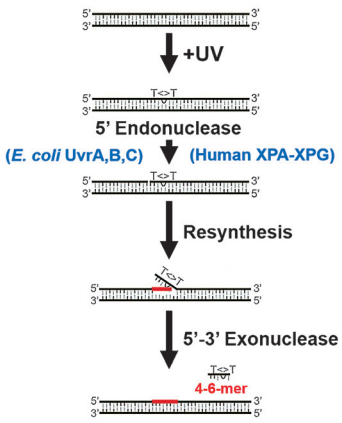
the T<>T by a cyclic redox reaction, and then the enzyme dissociates from the DNA to go on in search of other damage sites to carry out the repair reactions again.

Over the past decade we have collaborated with Dongping Zhong of Ohio State University to determine the microscopic rate constants of DNA repair by photolyase. We have determined the rates of energy transfer, electron transfer, bond breakage, bond forming and electron return, in real time and at picosecond resolution (Figure 6).[29–35] The entire catalytic cycle is complete in 1.2 ns, and the enzyme repairs T<>T with a quantum yield of 0.9.[29,31,34] Photolyase is currently one of the best understood enzymes.

Nucleotide Excision Repair

Excision Repair in E. coli

The work on photolyase, in addition to its intrinsic value, contributed to the discovery of the other major DNA repair mechanism found in nearly all cellular organisms: nucleotide excision repair (excision repair). In early work on photolyase, *E. coli* cells were irradiated with UV in a suspension in a buffer, and then one half was exposed to blue light while the other half was kept in the dark. It was found that the UV-induced T<>T dimers disappeared from the genome of the blue light-exposed cells, but remained unchanged in the genome of the control cells kept in the dark. However, if the same experiment was carried out in a buffer containing glucose as an energy source, incubation of UV-irradiated *E. coli,* in either the dark or the light, resulted in the disappearance of T<>T dimers from the genome.[36,37] Nevertheless, there was still a fundamental difference between the two sets of cells. In light-exposed cells, the T<>T dimers completely disappeared as expected. In contrast, in cells kept in the dark, even though the T<>T dimers disappeared from the genomic DNA, they accumulated quantitatively in the cytosol.[36–38] This finding in 1964 by Paul Howard-Flanders[36] at Yale University and Richard Setlow[37] at Oak Ridge National Laboratory led to the concept of nucleotide excision repair. After these initial findings, research done in numerous labs led to the conclusions summarized in Figure 7: T<>T dimers are removed (excised) from the genome in both *E. coli* and humans[39,40] in the form of 4 to 6 nucleotide (nt) long oligomers[36,37,40–42] but remain within the cell and are not exported. The excision reaction is genetically controlled by the *uvr* genes in *E. coli*[43] and *XP* genes in humans.[44,45] Following excision, the repair gap is filled in and ligated.[39,46] The consensus model for nucleotide excision repair over the period of 1964–1982 was the so-called "cut-and-patch" mechanism,[47] whereby an endonuclease controlled by the Uvr proteins in *E. coli*, and XP proteins in humans, made an incision 5′ to the T<>T, and then an exonuclease removed the T<>T in a reaction coupled with repair synthesis that filled in the single-stranded



- Thymine dimers are removed from the genome in both *E. coli* and humans.
- Excised thymine dimers were reported to exist in oligonucleotides 4-6 nt in length.
- Excision is genetically controlled by *uvr* genes in *E. coli* and *XP* genes in humans.
- Following excision, the repair gap is filled in and ligated.
- Excised dimers remain within the cell.

Figure 7. General model for nucleotide excision repair for *E. coli* and humans that was developed over the period of 1964–1982. It is referred to as the endonuclease/exonuclease coupled with repair synthesis or the cut-and-patch model.

gap, followed by ligation of the repair patch to complete the repair process.

In 1977, I joined the laboratory of Dean Rupp at Yale University to work on the mechanism of nucleotide excision repair in *E. coli*. I invented the maxicell method to specifically radiolabel plasmid-encoded proteins,[48] which enabled me to identify and clone the three genes implicated in excision repair: *uvrA*, *uvrB*, and *uvrC*.[49–51] Nothing was known about the specific functions of these genes at the time. I found that the three genes encoded proteins of 100 kDa, 85 kDa, and 66 kDa, respectively (Figure 8). With the aid of the maxicell method, I then purified the three proteins in milligram quantities (Figure 9) and investigated their effects on UV-damaged DNA. I found that the UvrA, B, C proteins repaired DNA by a mechanism different from the classic endonuclease/exonuclease (cut-and-patch) model: The three proteins instead act together to carry out concerted dual incisions at precise distances from the photoproduct,[52] seven nucleotides 5′ and three nucleotides 3′ from the damage to generate a dodecamer (12-mer) carrying the T<>T photoproduct. The excised oligomer is then removed from the duplex, and the resulting gap is filled and ligated (Figure 10). Later on at the University of North Carolina, my colleagues and I investigated the roles of the three proteins in the repair reaction. We found that UvrA and UvrB are ATPases and that UvrC is a nuclease, and we carried out detailed biochemical studies to develop the reaction mechanism shown in Figure 11:[53–68] UvrA recognizes the damage and recruits UvrB to the damage site, which promotes the formation of a stable UvrB–DNA complex in an ATP hydrolysis-dependent reaction. UvrA then disassociates from the complex, and UvrB recruits UvrC to the damage site. UvrC has two nuclease active sites, which make the 5′ and 3′ incisions in a concerted manner.[69,70] UvrC and the excised oligomer are then released from the duplex by the action of the UvrD helicase.[61] Finally, DNA polymerase I displaces UvrB and fills in the gap, and the repair patch is sealed by DNA ligase.[67]

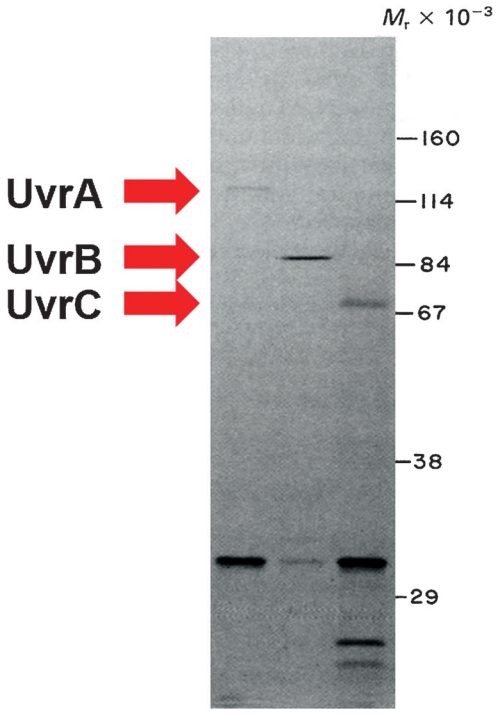


Figure 8. Identification of *E. coli* Uvr proteins. In the Maxicell method, a *recA*$^-$*uvrA*$^-$ mutant strain containing a plasmid carrying the gene of interest is irradiated with a moderate UV dose that hits the chromosomal DNA at multiple sites but not the much smaller (typically 500–1000 fold) plasmid. This causes total degradation of the chromosomal DNA in 6–12 hours leaving cells (maxicells) with only plasmid DNA. At this point, addition of the ^{35}S-methionine radiolabel to the medium labels only the plasmid encoded proteins which can be detected by autoradiography. This is an autoradiogram of three *E. coli* maxicells expressing UvrA, UvrB, and UvrC, respectively. The lower molecular weight bands are proteins encoded by the drug resistance genes, tetracycline and ampicillin. Reproduced from Ref. [48–51].

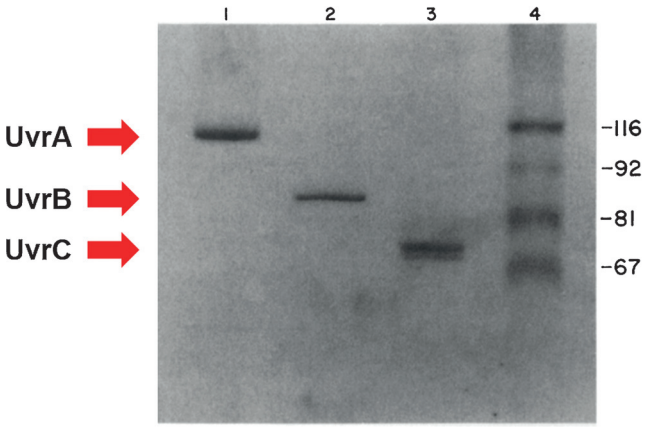


Figure 9. Purification of the UvrA, UvrB, and UvrC proteins. The proteins were purified from maxicells expressing the respective proteins, and the purification was monitored by radioactivity. The final purification products were analyzed by SDS-PAGE followed by Coomassie Blue staining. The last lane contains molecular size markers. Reproduced from Ref. [52].

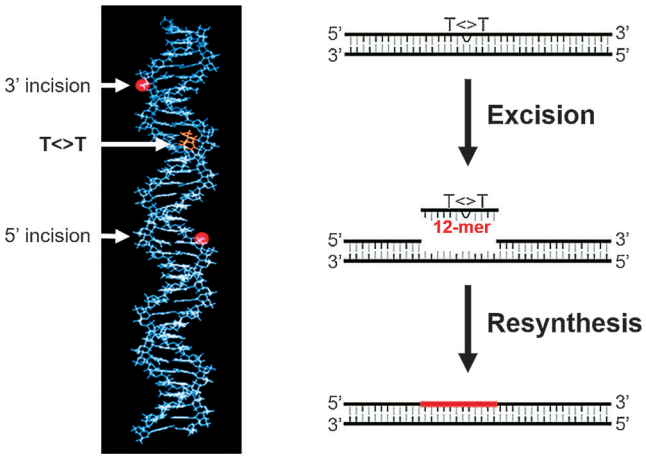


Figure 10. Excision by dual incisions in *E. coli*. UvrA + UvrB + UvrC proteins in the presence of ATP + Mg^{2+} incise 7 nucleotides 5′ and 3–4 nucleotides 3′ to the thymine dimer (T < > T) as shown in the 3D model (left) and line diagram representation (right). The 12–13 nucleotide gap is filled in by polymerases and ligated. Reproduced from Ref. [82].

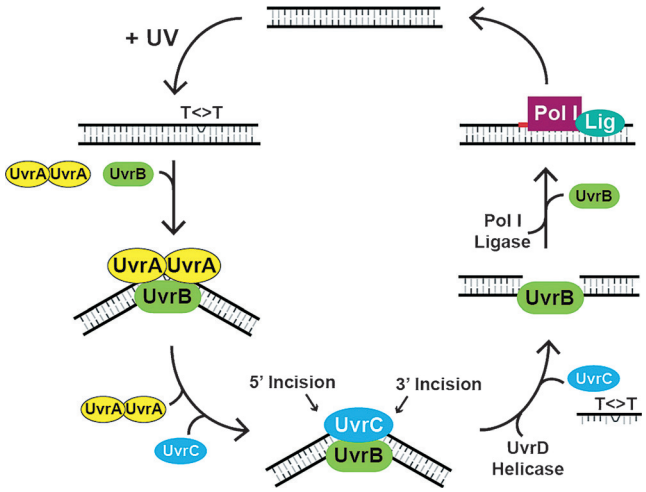


Figure 11. Reaction mechanism of excision repair in *E. coli*. The damage is recognized by the $(UvrA)_2$ homodimer which functions as a molecular matchmaker to recruit UvrB to the damage site. An ATP hydrolysis-dependent reaction then promotes the formation of a very stable UvrB–DNA complex. This complex recruits UvrC, which incises 5′ and 3′ to the damage due to active site nucleases within the N-terminal and C-terminal halves of the protein. UvrC and the excised dodecamer (12-mer) are then displaced by the UvrD helicase, and UvrB is displaced by DNA Polymerase I as it fills in the gap. The nick is then sealed by ligase. Reproduced from Ref. [65].

Transcription-Coupled Repair

While we were characterizing the reaction mechanism of the *E. coli* excision nuclease (excinuclease), Philip Hanawalt from Stanford University reported that transcription strongly stimulated repair in vivo in both mammalian cells and in *E. coli*.[71–73] It was proposed that this was the consequence of RNA polymerase enhancing the rate of the damage recognition, which is the rate limiting step in nucleotide excision repair. We therefore initiated a project to study the mechanism of *E. coli* transcription-coupled repair in a defined system using a damage DNA substrate, purified RNA polymerase, and the UvrA, B, and C proteins. We found that DNA damage blocked the progression of RNA polymerase, as predicted, and led to the formation of a very stable RNA polymerase elongation complex at the damage site. However, contrary to expectations that this stalled complex would accelerate repair by constituting a large target for the repair enzyme, we found instead that stalled RNA polymerase did not stimulate but actually inhibited repair, presumably by interfering with the access of UvrA, B, C to the damage site.[74] We reasoned that there must be a missing factor that performs two functions. First, it overcomes the repair inhibitory effect of stalled RNA polymerase, and second, it helps in recruiting UvrA, B, and C to the damage site to enhance the repair rate. We developed an in vitro biochemical assay to purify this coupling factor. We succeeded in purifying a protein of 130 kDa that performed both functions.[75–83] We named the protein transcription-repair coupling factor (TRCF). Using an entirely defined system, we elucidated the reaction mechanism of transcription-coupled repair in *E. coli* (Figure 12): TRCF is a translocase that recognizes stalled RNA polymerase and displaces it from the damage site while concomitantly recruiting UvrA to accelerate the repair rate. We also showed that TRCF was encoded by the *mfd* gene (mfd = mutation frequency decline) that was discovered by Evelyn M. Witkin in 1966 as a gene responsible for preventing a specific type of UV-induced mutagenesis. The discovery of the equivalency of TRCF and Mfd provided a mechanistic explanation for the *mfd*$^-$ phenotype that had remained mysterious for 25 years.[84]

Excision Repair in Humans

Having described the *E. coli* excision repair mechanism in some detail in 1987, we started to work on human excision repair. At that time, it was presumed that human excision repair proceeded by a 5′ endonuclease/exonuclease mechanism (cut-and-patch), as in the classical *E. coli* model[47] (Figure 7). Importantly, James Cleaver from the University of California San Francisco had discovered in 1968 that patients with the hereditary disease xeroderma pigmentosum (XP) were defective in excision repair.[44] These patients are extremely sensitive to sunlight and exhibit a circa 5000-fold increase in sunlight-induced skin cancer relative to individuals with normal excision repair (Figure 13). Genetic analysis of XP patients revealed that seven genes, termed *XPA* through *XPG*, were responsible for the removal of UV-induced photoproducts.[45] Using our expertise from working on *E. coli* excision repair, we proceeded to characterize human excision repair.[85] From the very beginning, we found that, as in *E. coli*, nucleotide excision repair was carried out in humans by dual incisions and not by the conventional model.[86–91] Beyond that, the human excision repair mechanism turned out to be very different from *E. coli* excision repair.[87–126] To begin with, in humans, not just three proteins (UvrA, B, C), but 16 proteins in six repair factors[93,97] were

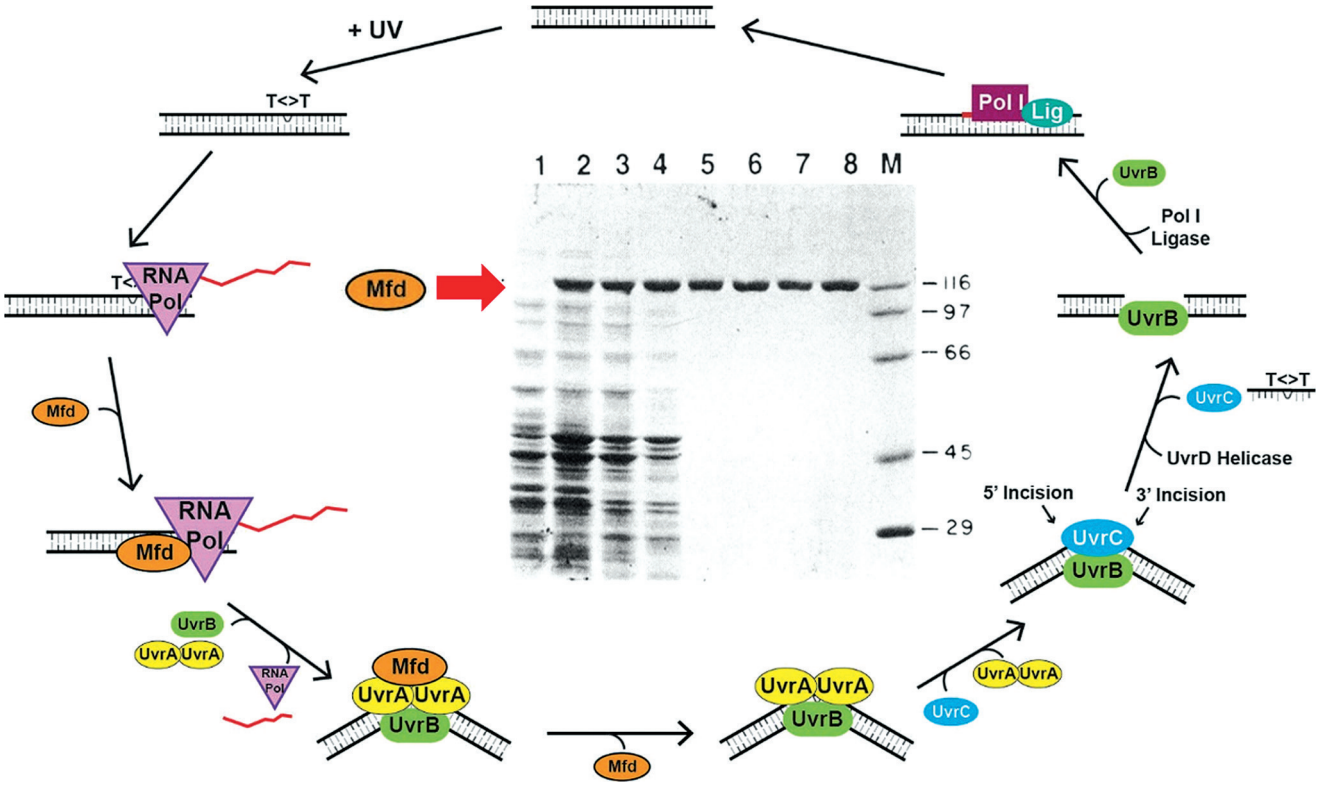


Figure 12. Molecular mechanism of transcription-coupled repair in *E. coli*. RNA polymerase stops when it encounters a T< >T in the transcribed strand of DNA. The ternary complex (DNA + RNA Polymerase + RNA) is stable for hours. The T< >T within this complex is not accessible to UvrA, and therefore repair is inhibited. The transcription-repair coupling factor (TRCF; also known as Mfd, mutation frequency decline) is a translocase (but not a helicase) with high sequence similarity to UvrB and affinity to RNA polymerase. Mfd (TRCF) recognizes the stalled complex and uses its translocase activity to displace RNA polymerase along with the truncated RNA. Because of its similarity to UvrB, Mfd simultaneously recruits UvrA, thus promoting the formation of a transient Mfd–UvrA–UvrB–DNA complex at the damage site and facilitating the rapid formation of the preincision UvrB–DNA complex, which is then followed by recruitment of UvrC and the dual incisions. The panel in the center shows the purification steps of Mfd (TRCF) from cells overproducing Mfd and analyzed by SDS-PAGE and Coomassie Blue staining. Reproduced from Ref. [77].

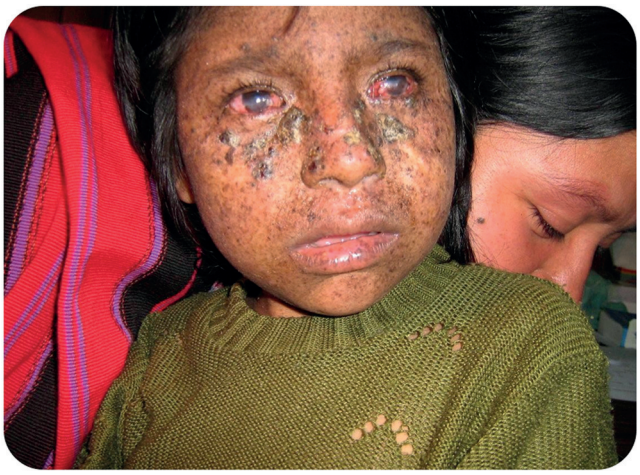


Figure 13. A Xeroderma pigmentosum patient. The patients in complementation groups A–G are very sensitive to sunlight and even regular white light from electric light sources. XP patients exhibit a several thousand-fold higher incidence of skin cancer compared to normal individuals. Reproduced from: J. Halpern et al., *Cases J.* **2008**, *1*, 254.

necessary for making the dual incisions (Figure 14). Furthermore, these human proteins are not evolutionarily related to the *E. coli* excision repair proteins. Secondly, even though in principal, both *E. coli* and humans carry out nucleotide excision repair by dual incisions, the dual incision mechanisms are quite different. Whereas in *E. coli* the 5′ incision is seven nucleotides away from the damage, and the 3′ incision is three nucleotides away from the damage, the human excision repair system incises the damaged strand 20–22 nucleotides 5′ and five nucleotides 3′ to the damage to release an excised oligomer of 27–30 nt in length,[86] in contrast to the 12–13 nt oligomer generated by *E. coli* dual incisions (Figure 15). Finally, the actual damage recognition and processing is also different: Whereas damage is recognized by UvrA in *E. coli*, damage is instead recognized in humans by RPA, XPA, and XPC, followed by recruitment of TFIIH, which contains the XPB and XPD helicases that unwind the helix and recruit the XPG and XPF nucleases to make the 3′ and 5′ incisions. The dual incision event is followed by the release of the 30-nucleotide excised oligomer, gap filling and ligation by DNA polymerase and ligase to produce a 30-nucleotide repair patch.[102] Figure 16 summarizes our current model for the mechanism of human nucleotide excision repair. Most

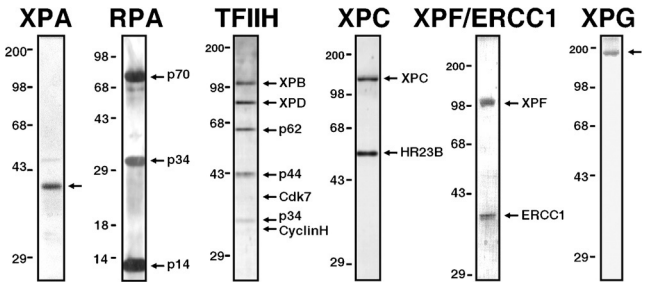


Figure 14. Human excision repair factors. Six repair factors encompassing 16 proteins are needed for making the dual incisions in humans. Note that RPA also functions in replication and recombination. TFIIH, which contains eight other subunits in addition to the XPB and XPD helicases encoded by the respective XP genes, is a general transcription factor for the initiation of transcription by RNA Polymerase II. These human excision repair proteins are not evolutionarily related to prokaryotic excision repair proteins. The figure shows purified repair factors separated by SDS-PAGE and silver stained. Reproduced from Ref. [93, 97].

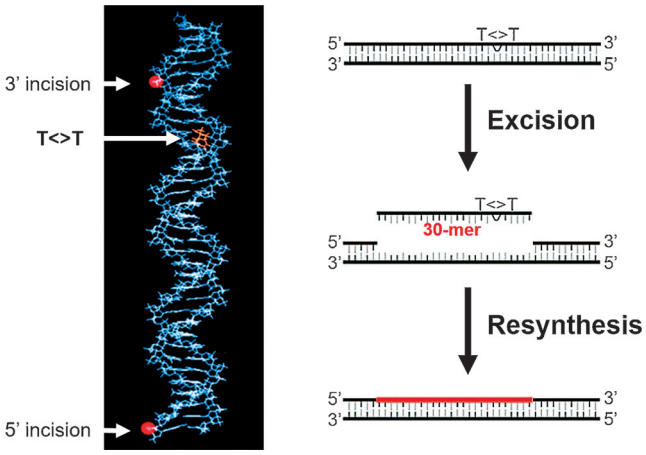


Figure 15. Excision by dual incisions in humans. In humans, thymine dimers (T<>T) and other bulky base adducts are removed by dual incisions located 20±5 phosphodiester bonds 5′ and 6±3 phosphodiester bonds 3′ to the damage, which releases an oligonucleotide 24–32 nt in length (referred to as nominal 30-mers). Left: Dual incision sites on a 3D representation of DNA. Right: Schematic of human dual incisions followed by repair synthesis and ligation. Reproduced from Ref. [82, 86].

recently this work led us to study other cellular responses to DNA damage including the DNA damage checkpoints.[127–130]

Excision Repair Map of the Human Genome at Single Nucleotide Resolution

Our discovery of excision of a nominal 30-mer by the human nucleotide excision repair system in cell extracts and with the reconstituted enzyme system was confirmed by other groups. However, these findings were at odds with numerous reports that the excised T<>T dimers were in the form of oligomers 4 to 6 nucleotides in length in human cells.[40–42] This discrepancy between the in vivo and in vitro data remained

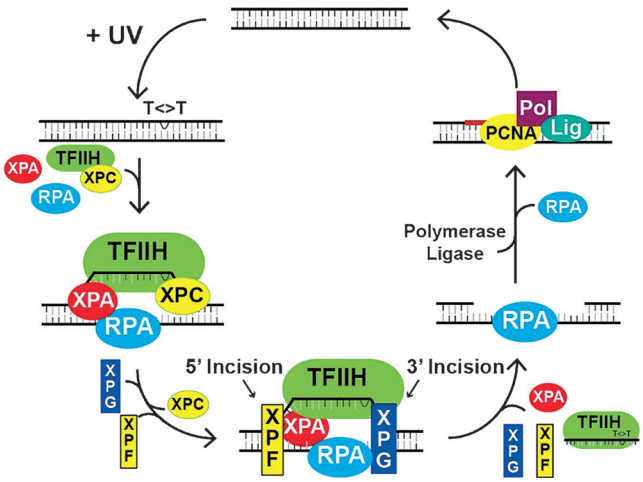


Figure 16. Reaction mechanism of the human excision nuclease system. The damage is recognized by cooperative interactions of RPA, XPA, and XPC followed by recruitment of TFIIH. The helicase activity of TFIIH provides the major specificity by kinetic proofreading and results in formation of a tight complex from which XPC is ejected. Note that XPC plays a role similar to that of *E. coli* UvrA by playing an essential role in damage recognition leaving the complex to allow subsequent steps to proceed (molecular matchmaker). Concomitant with the dissociation of XPC, the XPG and XPF nucleases are recruited to make the 3′ and 5′ incisions in a concerted reaction. The excised "30-mer" is released in a tight complex with TFIIH. The excision gap is filled in by DNA polymerases and ligated to produce a 30 nt long repair patch. Reproduced from Ref. [116].

unresolved for two decades. The solution to the problem came from the analysis of the fate of the excised 30-mer in vitro using cell-free extracts and reconstituted repair reactions. We found that the excised 30-mer released from the duplex is in a tight complex with TFIIH in in vitro reactions.[131,132] We reasoned that this may be the case in vivo as well. To test this prediction, we irradiated human cells and after incubating for 1–6 hours to allow for repair, we lysed the cells, immunoprecipitated TFIIH, and analyzed the DNA fragments associated with TFIIH. We found that the excision products generated in vivo were in fact 30 nucleotides in length, as in the case of the in vitro reaction.[133–137] Upon longer incubation, the primary excision product is degraded to smaller fragments less than 10 nucleotides in length, which explained the previous in vivo studies in which the excised oligonucleotides were typically isolated from the cells 24 hours after irradiation.

Our ability to isolate the primary excision product not only solved the apparent discrepancy between the in vivo and in vitro excision reactions, it also provided a means for generating a repair map of the entire human genome (Figure 17).[138] Following irradiation of cells with UV and incubation for a period of time to allow for repair, we then lysed cells and immunoprecipitated TFIIH to isolate the associated excised oligomers. The excised oligomers are then sequenced using next generation sequencing (NGS). In a typical experiment we obtain 15–20 million reads. We align these reads to the human genome to place all of the excision products at specific locations thus generating a repair map. Figure 18 shows the repair map of the 22 somatic and 2

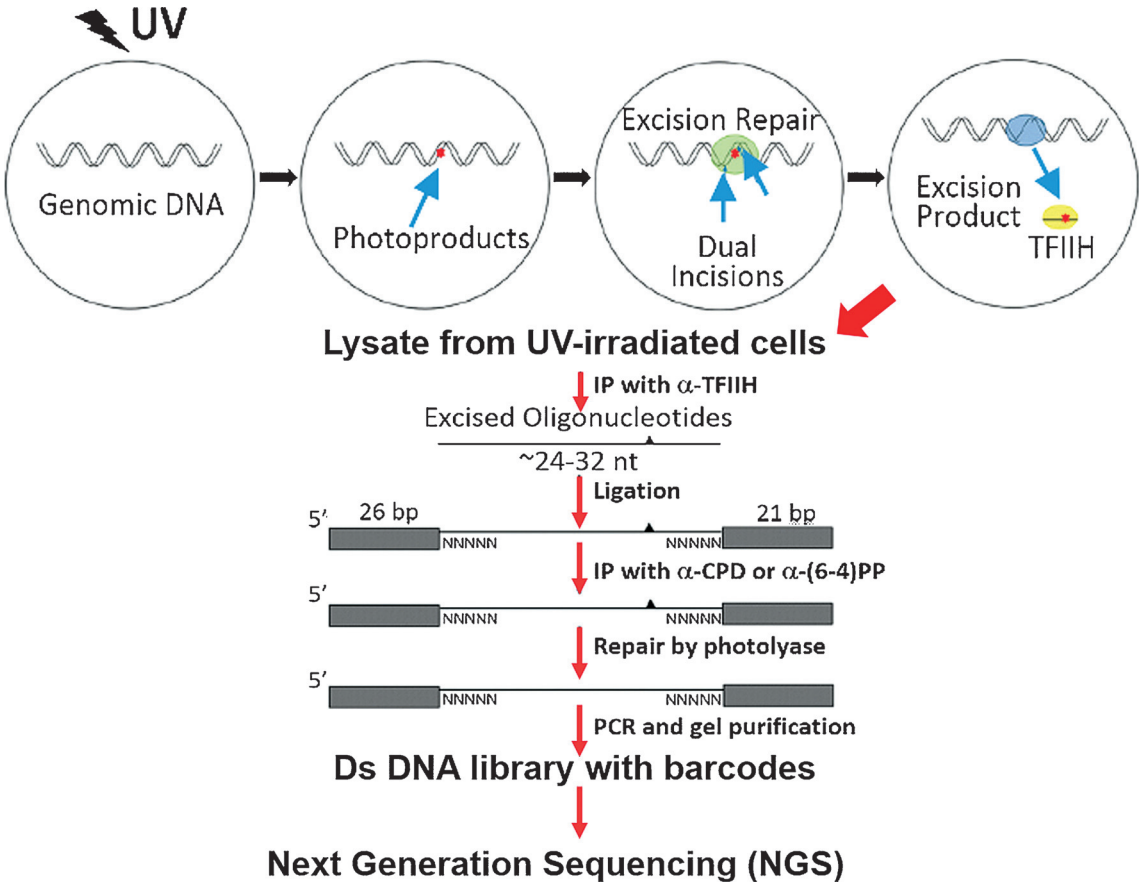


Figure 17. The XR-seq (excision repair-sequencing) method for generating the human excision repair map. UV-irradiated cells are lysed, following an incubation period, and TFIIH is immunoprecipitated. The excised nominal 30-mers (24–32 nt) which co-precipitate with TFIIH are extracted and ligated to adaptors. The oligomers containing cyclobutane pyrimidine dimers (CPD) or (6–4) pyrimidine-pyrimidone photoproducts are then immunoprecipitated with the corresponding photoproduct antibodies. The photoproducts are repaired by the appropriate photolyases, and the oligomers are amplified by PCR, gel purified, and subjected to sequencing by next generation sequencing (NGS) using the Illumina HiSeq 2000 platform. Reproduced from Ref. [138].

sex chromosomes of a male individual. The black tracks represent transcription and the green tracks represent the repair tracks of the two photoproducts for the two strands of the entire genome. This figure is a screenshot of the repair map of the entire genome, and is meant to illustrate the coverage of repair over the whole genome. However, it does not reveal much information about the determinants of repair mode and rate at a given locus. By concentrating on one specific chromosome at various resolutions, the information contained within this map becomes apparent. Figure 19 shows the repair map of chromosome 17. This chromosome is 83 megabases in length and carries the *p53* gene, which is mutated in about 50% of human cancers. The map shows the transcripts along the entire chromosome in both strands in black and the excision repair (XR-seq) tracks for both strands in blue. The repair map is a map in the true, geographic sense of the word. It has mountains, it has valleys, and it has canyons, meaning there are regions of high repair, low repair and no repair at all. Importantly, with such a map, we can answer the question of the repair mode and efficiency at any given nucleotide in the genome. As an example, Figure 19 shows the *p53* transcription and repair maps at kilobase resolution (middle). Finally, at single nucleotide resolution (bottom) the map shows the repair efficiency and the mode of repair of a *p53* mutation hotspot at T–T (7,577,150–7,577,151) dinucleotide position. The thymine dimer at this position is removed by incision 20 nucleotides 5′ and 4 nucleotides 3′ to the photoproduct. It is evident that much more information can be gathered from this map regarding the determinants of repair of UV damage at any given location of the genome. More importantly, nucleotide excision repair also repairs the DNA damage caused by the major anticancer drug, cisplatin. We are currently generating a cisplatin damage repair map of the genomes of normal and cancerous human cells that we hope will have some implications for cancer treatment.

To summarize (Figure 20) our work on nucleotide excision repair:[67,68,82,83,120,121,124] Repair is initiated by dual incisions both in *E. coli* and in humans, which generates 12–13-mers in *E. coli* and approximately 30-mers in humans. The dual incisions require UvrA, B, and C proteins in *E. coli* and six repair factors encompassing 16 proteins, including the proteins encoded by the *XPA* through the *XPG* genes, in humans. Following excision, the gap is filled in by DNA polymerases and ligated to generate repair patches of 12–13 and approximately 30 nucleotides in *E. coli* and humans, respectively. Finally, by capturing the excised 30-mer gener-

Figure 18. Excision repair map of the entire human genome. The locations of the XR-seq signals for CPD and (6–4)PP in both strands of the duplex across all chromosomes of the human NHF1 cell genome (male) are indicated by green tracks. The ENCODE total stranded RNA-seq tracks in black are plotted on top of the XR-seq tracks for comparison. Chromosome 17, which is boxed in red, carries the *p53* gene which is mutated in nearly 50% of cancers. Reproduced from Ref. [138].

ated by human nucleotide excision repair in vivo, we have generated the excision repair map of UV damage of the whole human genome.

Cryptochrome, Circadian Clock, and Closing the Circle

I wish to conclude this presentation by explaining how our work on photolyase led to the discovery of cryptochrome as an essential component of the mammalian circadian clock, and how the circadian clock regulates nucleotide excision repair in mammals, thus linking the two subjects of our long-term research projects, photolyase and nucleotide excision repair.

Discovery of Mammalian Cryptochrome

I have discussed the excision repair mechanisms in both humans and *E. coli*, although I only presented photolyase data for *E. coli*. This is because humans do not have photolyase.[139] In fact, for 30 years after the discovery of photolyase in *E. coli*, its presence in humans was a matter of controversy. Some investigators reported that photolyase was not detectable in human cell lines, while others reported robust photolyase activity in human cells and reported purification of the enzyme to homogeneity from human white blood cells.[140] Having developed very sensitive and specific assays for photolyase in the 1980's, we decided to resolve this controversy. We conducted a comprehensive search for photolyase in freshly isolated human white blood cells. We detected no photolyase activity and then published a paper in 1993[139] categorically stating that humans do not have photolyase (Figure 21). However, 2 years later, in one of the first public releases of the human genome project, one of the ESTs (expressed sequence tags) was listed as the photolyase homolog.[141] Reasoning that we may have missed the photolyase activity in our earlier work, we decided to investigate the function of the gene by obtaining the entire cDNA clone, expressing it, and analyzing its function. While this work was in progress we discovered a second photolyase "homolog" in the human genome. We obtained the entire cDNAs of both genes. They are remarkably similar to *E. coli* photolyase at the sequence level and, equally remarkable, at the 3D structure level (Figure 21). We expressed and purified the proteins encoded by these genes and established that they had no photolyase activity and concluded that they were photo-

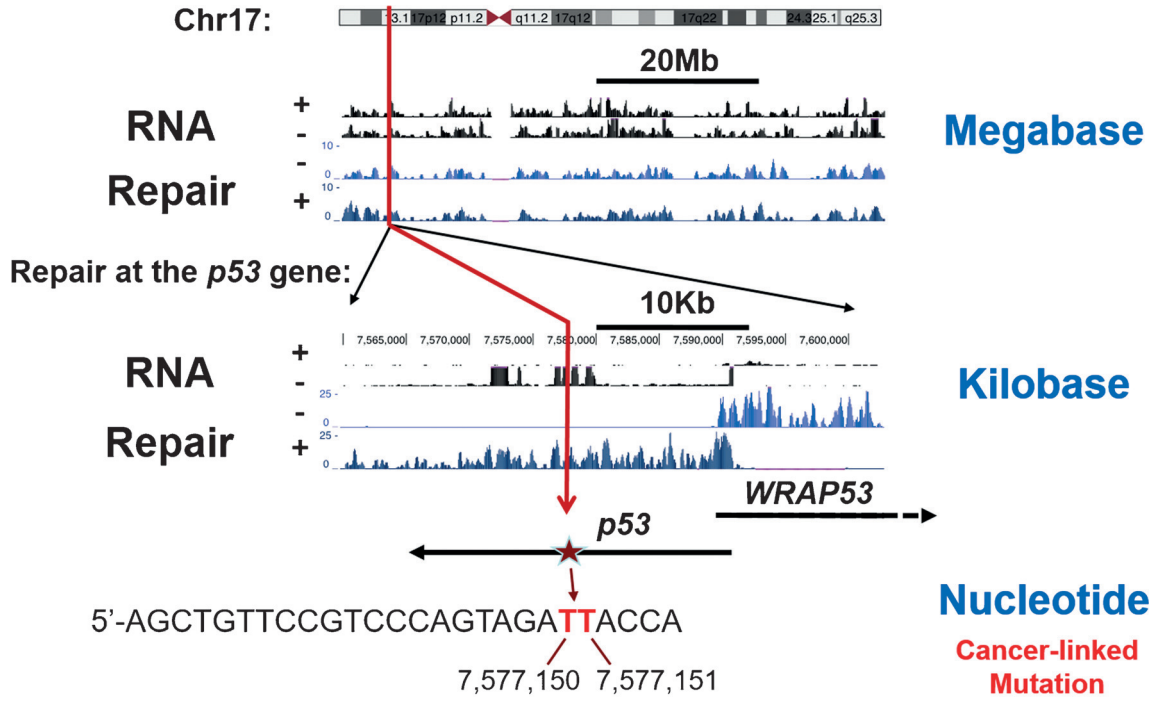


Figure 19. Excision repair at single nucleotide resolution. The transcription and repair maps of chromosome 17 are shown in an XP-C mutant cell line, which can only carry out transcription-coupled repair, to illustrate the dramatic effect of transcription on repair. The red line indicates the position of the mutation hotspot in the *p53* gene. Top: Transcription and repair map at megabase resolution. Middle: Transcription and repair maps at kilobase resolution. Note the strong repair signal in the transcribed strands of the *p53* and *WRAP53* genes with a nearly absolute lack of repair in the non-transcribed strand. Bottom: The repair pattern of T<>T dinucleotide at a mutagenic hotspot, position 7,577,150–7,577,151. The photodimer is removed in the form of a 26-mer by dual incisions 20 nucleotides 5′ and 4 nucleotides 3′ to the dimer. Reproduced from Ref. [138].

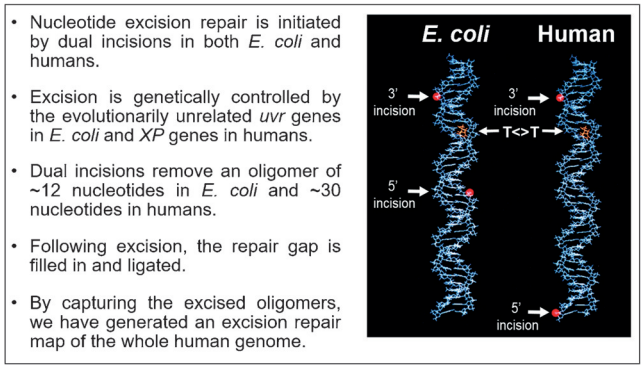


Figure 20. Excision repair in *E. coli* and humans. In both organisms, excision is by dual incisions. However, the proteins required for the dual incisions, the mechanisms for damage recognition, and the dual incision patterns are entirely different.

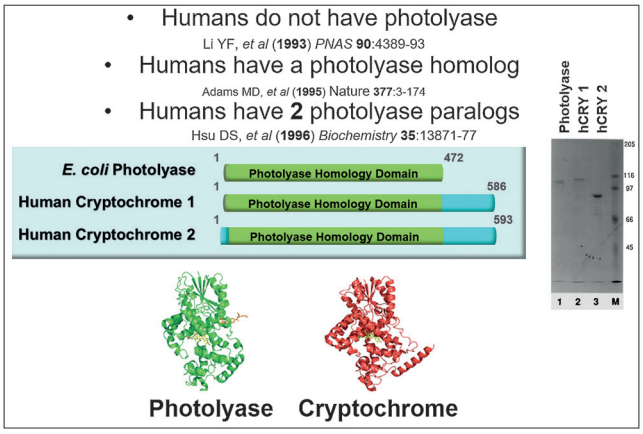


Figure 21. Photolyase–cryptochrome connection. Top: Key papers leading to the discovery of the human cryptochromes. Middle: Sequence similarities among *E. coli* photolyase and human cryptochromes 1 and 2. Bottom: Three-dimensional structures of *E. coli* photolyase and *Arabidopsis* cryptochrome 1 showing the similarities in the photolyase homology domain. Reproduced from: C. A. Brautigam et al., *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12142–12147. Side panel, reproduced from Ref. [144]: Purified *E. coli* photolyase and hCRY1 and hCRY2 analyzed by SDS-PAGE followed by Coomassie Blue staining.

lyase paralogs. This work was completed in April 1996, and not knowing what functions of these paralogs might be, we were reluctant to publish our findings (Figure 22). In May 1996, I made my annual pilgrimage to Turkey to visit my family. On my return trip I read an article on jetlag in the airline magazine entitled "Internal Timekeeping," by William Schwartz.[142] I believe this was the first time I learned the meaning of the phrase "circadian clock." The article, among other things, noted that the circadian clock was synchronized to the physical clock by light, and was particularly sensitive to blue light. After reading this article, I suspected that the human photolyase paralogs might be clock proteins that sense blue light. Upon returning to the lab, I suggested to my co-

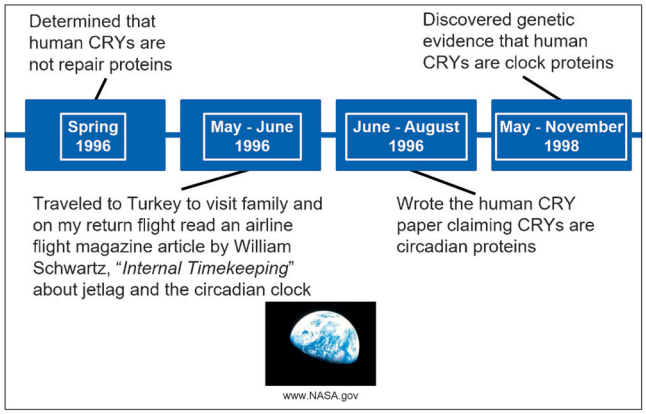


Figure 22. Sequence of events leading to discovery of human CRY as a circadian clock protein. Although the prediction was that CRY was the blue light sensor of the mammalian clock, genetic analysis revealed it to be an essential cog in the core clock machinery.

workers that we publish our data and that we name the human photolyase paralogs cryptochromes in analogy with the plant blue light photoreceptors with sequence similarity to photolyase.[143] I also proposed that we suggest that the human cryptochromes (CRY1 and CRY2) are circadian clock proteins.[144] We wrote the paper and submitted it for publication in August, and it was published in November 1996.[144] We then proceeded to test this claim[145] by knocking out the *CRY* genes in mice and testing them for circadian clock function.

The Circadian Clock

Before presenting the data on the mouse *CRY* knockouts, I will briefly summarize what the circadian clock is (Figure 23):[146–149] The clock, in general, is a timekeeping object/system. The circadian clock is similar to the clocks we are familiar with, including mechanical and electrical clocks that are based on mechanical and electronic principles. The circadian clock has the same kind of design except that the

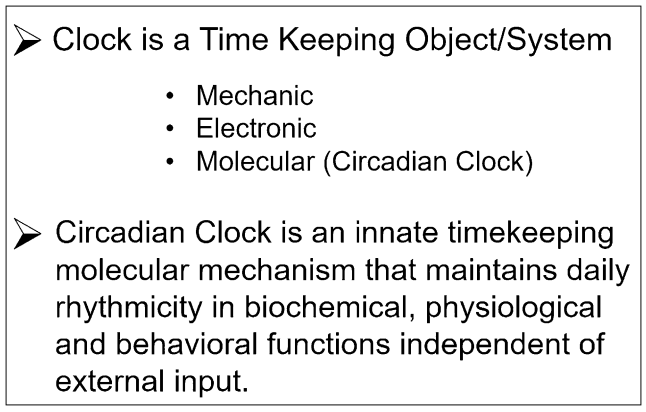


Figure 23. Clock and circadian clock. The circadian clock, like the mechanical and electronic clocks, measures time independent of external stimuli, but it is instead made up of molecules rather than gears and levers or electronic circuits.

components that make up the clock are molecules, and the function of the clock is to inform us of the time of the day. By doing so, the circadian clock maintains daily rhythmicity in biochemical, physiological, and behavioral functions of the organism even in the absence of external input.

Role of Cryptochrome in the Circadian Clock

To test whether CRYs are circadian clock proteins, we generated mice with mutations in either *CRY1* or *CRY2*, or both, and analyzed their circadian clock by recording their daily wheel running activity for 28 days.[150,151] For the first week the mice were kept under 12 hours of light and 12 hours of dark (LD12:12), and for the final 3 weeks they were kept in constant darkness. The results are shown in Figure 24. Under these light:dark conditions, wild-type (WT) mice and mutant

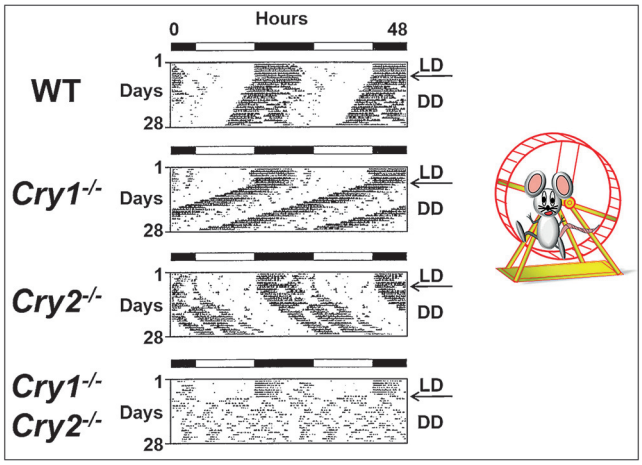


Figure 24. Behavioral analyses of cryptochrome knockout mice. Mice of the indicated genotypes were kept in cages with running wheels for 28 days and their activity profiles were recorded (actogram). The rpm of the running wheel is plotted on the *y* axis, and the time of the day is plotted on the *x* axis. The bar on top indicates the dark and light phases of the day. On the 7th day, indicated by arrows, the mice were switched from 12 h light:12 h dark (LD) cycle into constant darkness (DD). Note that under LD all 4 mice exhibit similar activity and rest phases with a 24 h periodicity. In DD, the mice exhibit activity/rest phases with periodicities imposed by their intrinsic clock: wild type 23.7 h; $Cry1^{-/-}$ 22.7 h; $Cry2^{-/-}$ 24.7 h. The $Cry1^{-/-}$ $Cry2^{-/-}$ double knockout is arrhythmic because it has no functional circadian clock. Reproduced from Ref. [150,151].

mice were active during the dark and rested during the day, as would be expected because mice are nocturnal animals. However, under conditions of continuous darkness with no external stimuli, the mice behaved differently. Whereas, the WT mouse exhibited an activity–rest rhythm with a periodicity of 23.7 hours, the *CRY2* mutant exhibited a longer period of 24.7 hours and the *CRY1* mutant had a rhythmicity with a period of 22.7 hours. More strikingly, the $CRY1^{-/-}$ $CRY2^{-/-}$ double knockout totally lost rhythmic behavior in constant darkness. These findings established cryptochromes as core clock proteins. While this work was going on in our lab,[152–170] there was a great deal of scientific discoveries in the

circadian field over the period of 1996–2000 that led to the identification of the four classes of proteins (Figure 25) that are essential for controlling the circadian clock in humans.[146–149,171,172] The following model was developed for the molecular clock: CLOCK and BMAL1 activate the transcription of CRY and PER, which after a time delay, enter the nucleus and inhibit their own transcription resulting in rise and fall of CRY and PER levels with a periodicity of about 24 hours. In addition, these core clock proteins control the expression of about 30% of all genes in a given tissue to confer this cyclic expression pattern and thus a daily rhythmicity of functions (Figure 26).

1) CRYPTOCHROME (Flavoprotein)
2) PERIOD (PAS domain)
3) CLOCK (bHLH-PAS)
4) BMAL1 (bHLH-PAS)

Figure 25. Mammalian clock genes and proteins. The 4 genes and their paralogs were cloned, and the proteins were characterized over the course of 5 years.

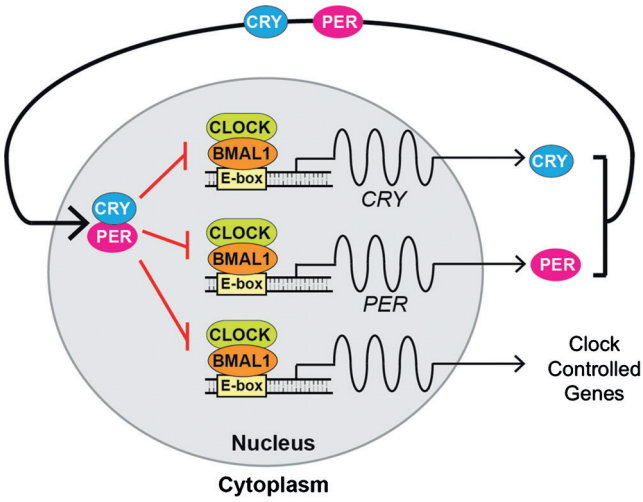


Figure 26. Molecular mechanism of the mammalian circadian clock. CLOCK and BMAL1 are transcriptional activators, which form a CLOCK–BMAL1 heterodimer that binds to the E-box sequence (CACGTG) in the promoters of *Cry* and *Per* genes to activate their transcription. CRY and PER are transcriptional repressors, and after an appropriate time delay following protein synthesis and nuclear entry, they inhibit their own transcription, thus causing the rise and fall of CRY and PER levels with circa 24 hour periodicity (core clock). The core clock proteins also act on other genes that have E-boxes in their regulatory regions. As a consequence, about 30% of all genes are clock controlled genes (CCG) in a given tissue, and hence exhibit daily rhythmicity. Among these genes, the *Xpa* gene, which is essential for nucleotide excision repair, is also controlled by the clock. Reproduced from Ref. [170].

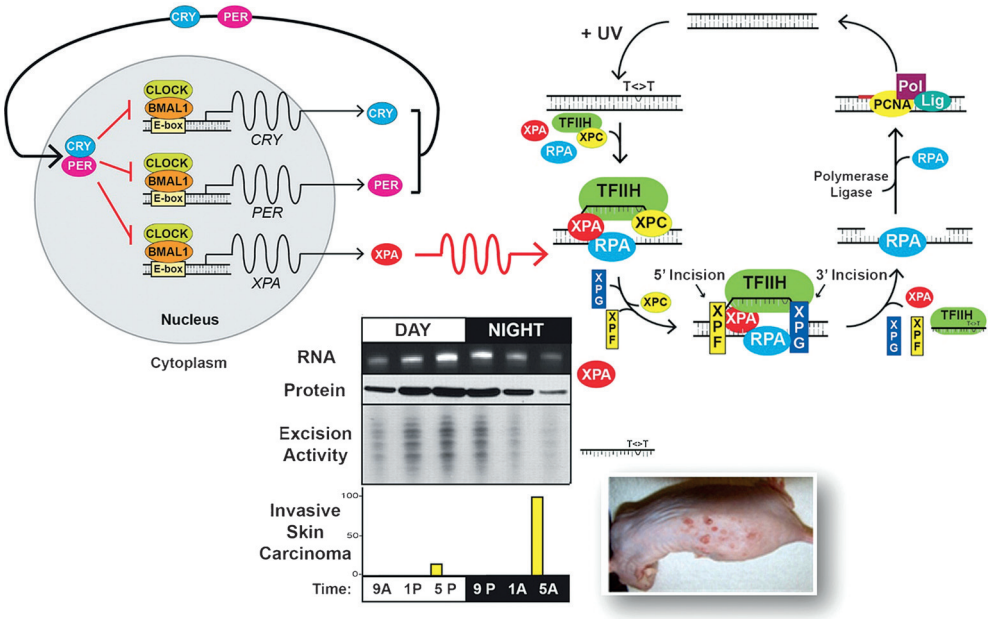


Figure 27. Circadian control of excision repair and photocarcinogenesis in mice. The core circadian clock machinery controls the rhythmic expression of XPA, such that XPA RNA and protein levels are at a minimum at 5 am and at a maximum at 5 pm. The entire excision repair system therefore exhibits the same type of daily periodicity. As a consequence, when mice are irradiated with UVB at 5 am they develop invasive skin carcinoma at about 5-fold higher frequency compared to mice irradiated at 5 pm when repair is at its maximum. The mouse in the picture belongs to the 5 am group with multiple invasive skin carcinomas at the conclusion of the experiment. Reproduced from Ref. [174,176].

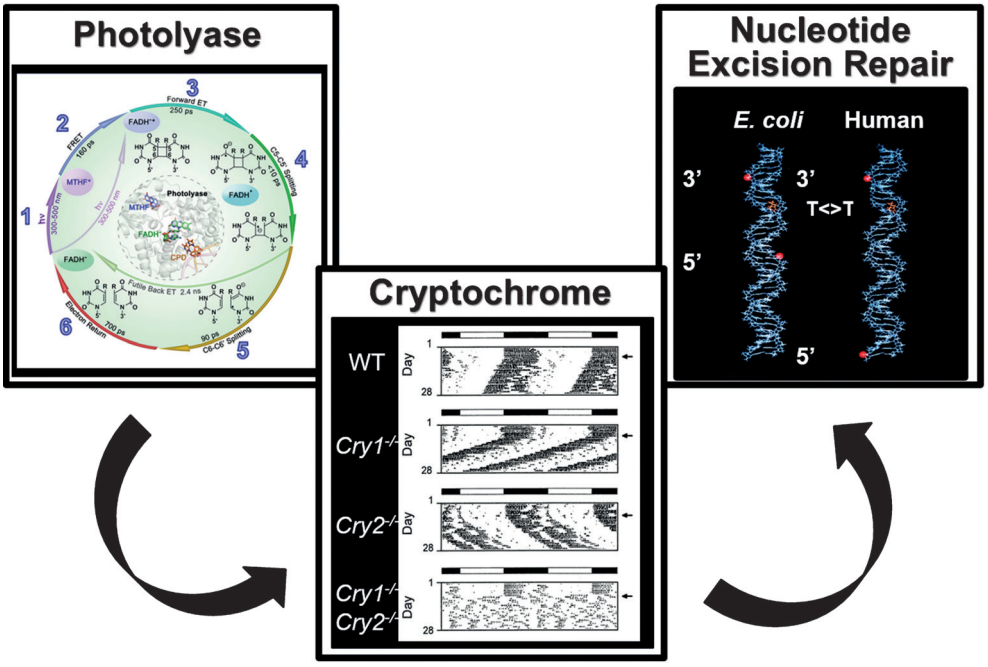


Figure 28. Photorepair, cryptochrome, and nucleotide excision repair. I have worked for over 40 years on photolyase, whose photocycle is shown here. I have spent 35 years on nucleotide excision repair and discovered two different dual incision patterns in *E. coli* and humans as shown. The photolyase work eventually led to the discovery of cryptochrome as a core clock protein, as exemplified by these actograms. The core clock controls nucleotide excision repair in mice which revealed that a photolyase-like protein (cryptochrome) with no repair activity nevertheless controls repair through the circadian clock.

Circadian Clock, Control of Excision Repair, and Carcinogenesis

Among the genes regulated by the circadian clock, we found that the excision repair protein XPA is controlled by the biological clock, and we therefore asked whether the entire nucleotide excision repair oscillates with daily periodicity. As shown in Figure 27, XPA transcription and protein levels are at a maximum at around 5 pm and at a minimum at around 5 am. Importantly, the entire excision repair activity shows the same pattern.[173–175] This led to the prediction that mice would be more sensitive to UV light when exposed at 5 am (when repair is low), compared to 5 pm (when repair is high). We proceeded to test this prediction. We irradiated two groups of mice with UV at 5 am and 5 pm, respectively, and found that the group irradiated at 5 am exhibited 4–5 fold higher incidence of invasive skin carcinoma than the group irradiated at 5 pm.[176] Currently, we are investigating whether this rhythmicity of excision repair exists in humans, and if it does, whether it can be used to make public health recommendations to prevent skin cancer.[170,177] Equally important, excision repair is also the repair mechanism for the DNA damage caused by the anticancer drug cisplatin. We are thus also investigating whether this periodicity of excision repair can be used to improve cisplatin treatment in cancer.

Summary

To conclude, Figure 28 is the summary of my 40 years of work on photolyase, 35 years of work on nucleotide excision repair in *E. coli* and humans, and 20 years of work on photolyase-related cryptochrome that links these two repair pathways that I have worked with all my career.

Acknowledgements

I have had the good fortune of having worked with outstanding students and postdocs over the course of my career who have conducted most of the experiments I described here (Figure 29). I am grateful to my internal medicine professor, Muzaffer Aksoy, who encouraged me to go to the United States to do research. My mentor, Dr. Rupert, discovered DNA repair in the modern sense, and he

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Figure 29. Sancar lab members.

Mentors

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Funding

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Figure 30. Mentors and collaborators.

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Photolyase	Circadian Clock	Excision Repair	
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Figure 31. Contributors.

has been my role model throughout my scientific career. W. Dean Rupp and Paul Howard-Flanders introduced me to nucleotide excision repair and helped lay the scientific foundation for my research on DNA repair (Figure 30). I have been very fortunate to have had collaborators who were leaders in the field of DNA enzymology, flavin photochemistry, analytical chemistry, crystallography, ultrafast chemistry, and the mammalian circadian clock (Figure 30). Finally, I wish to acknowledge the scientists who have made important contributions in the fields of photolyase, excision repair, and circadian clock research. In this lecture I was not able to fully reference all contributions to these fields, but have tried to place our work in historical context. Science is not done in a vacuum. We have greatly benefited from work done on these topics by our predecessors as well as our contemporaries who carried out similar work on these topics (Figure 31). Their ideas, findings, and shared reagents have been critical to the success of my laboratory. I wish to acknowledge Laura Lindsey-Boltz, Michael Kemp, and Rita Meganck for their assistance preparing this lecture and manuscript.

Biography

Introduction

"On 11 March 1890, a five-hour banquet for hundreds of invited guests was held in the festive chamber of the Berlin City Hall. A festival of a magnificence perhaps unparalleled in the history of science … The vast chandeliered room was decorated with palm trees and laurel leaves, and one end was dominated by a five-metre-high oil painting of Bismarck and other European statesmen carving up the Turkish empire at the Congress of Berlin."

I first read this paragraph in 2004, in John Buckingham's excellent book about the history of chemistry, *Chasing the Molecule*.[178] The banquet was in honor of Kekule, who is the main formulator of the theory of chemical structure (the theory that all molecules have definitive 3-dimensional structures) and whose discovery of the hexagonal molecular structure of benzene in 1865 was a major breakthrough in both pure and applied chemistry. I was impressed by the central theme of the Benzolfest and the celebration of chemistry, and of science in general, but also struck by the cavalier attitude of the Europeans of that period, and apparently of the author about "carving up the Turkish Empire". These two subjects, science and the Turkish Nation (Ottoman Empire and Republic of Turkey), not necessarily in that order, have dominated my thinking for as long as I can remember. I grew up as, and still am, a Turkish patriot and, from the age of about 10 I was also an aspiring, and later practicing, scientist.

The Early Years

I was born on September 8, 1946, in a small town named Savur, in the Mardin Province of southeastern Turkey, the seventh of eight children of Abdulgani and Meryem Sancar (Figure 32). I also had two half-brothers. Father was a farmer, and Mother took care of the children and the house. By the standards of the day we were a lower middle-class family. We always had enough to eat, but shoes were luxuries, and until the seventh grade we wore them only when we went to school. Much of my early youth was spent in the valley below our house where, alongside my brothers and father, I tended the fruit and nut trees and the vegetable garden that provided our family nourishment and income. We also had a few farm

Figure 32. Meryem Sancar pregnant with Aziz in 1946 in Savur, Turkey. The little girl in the background is the 4-year old Seyran, Aziz' youngest sister.

animals that provided milk and meat for our family throughout the year. My most pleasant memories from childhood are the flowering of the almond and plum trees in our orchard in the spring. In those early years, I began to learn about Islam and was convinced that Paradise must look like our orchard when the almond trees were in full bloom.

Overall, I did not like farm work. The terraces in the vegetable garden were held in place by stone walls constructed without mortar and required constant maintenance by me and my brothers. Walnut harvesting was hard work, and as one of the younger children, I had to climb very high into the trees to make sure all the walnuts fell. But the worst was herding baby goats because they could run faster than any 5–7 year old boy. My younger brother and I were in charge of herding them and spent many terrified hours trying to find the runaways before Father noticed they were gone.

Our large extended family (Figure 33) was an important part of my early childhood. Uncles, aunts, and many cousins lived in Savur and there were often other relatives visiting from towns farther away. Visits with my Uncle Sevket and his family in Mardin City were another high point. Mardin is known for its beautiful architecture which dates primarily from 1100–1300 A.D. Sleeping in large beds on the rooftop of Uncle's house was always a treat. As I fell asleep, I would watch on the horizon the lights of two nearby Syrian towns, and in the morning I would wake to the call to prayer from the historic Sehidiye Mosque about 200 meters from our house.

Early Influences

The three most important influences in my early education, in addition to Mustafa Kemal Ataturk, were my mother Meryem, my father Abdulgani, and Kenan, my oldest brother (Figure 34). Beginning in 1911, and until the end of the Turkish War of National Liberation in 1922, the Ottoman Empire was in a constant state of war trying to prevent the "carving-up of the Turkish empire" by the Europeans, leaving the country economically exhausted and decimated due to the loss of much of its most productive lands and populations. During this time of turmoil and economic hardship, many in my grandparents' and parents' generation did not have the opportunity to obtain even an elementary education. Mustafa Kemal Ataturk led and won the War of Turkish National War of Liberation against the occupying European forces, a war that gave rise to the modern Turkish Republic. The new Republic gave priority to developing an education system available to all Turkish citizens. In a short period, schools were opened throughout the country, manned by teachers who were committed to Ataturk's vision of an educated citizenry, idealistic about their country and optimistic about Turkey's future. As a result, unlike my parents and grandparents, even in an underdeveloped, rural part of Turkey I had access to excellent teachers and an excellent education that instilled in me pride in the history of the Turkish people and confidence that we could accomplish great things.

My mother was an illiterate woman who was the daughter of an Imam in a small village near Savur. Although she could not read or write, she was the most intelligent woman I have known. She was also very progressive and virtually worshipped Ataturk. It was at her insistence that all of her children got some degree of education. My father was the hardest working man I have ever known. He was, and still is, my role model. My oldest brother, Kenan, taught me how to read and write when I was 5 years old. Therefore, when I began school I was well ahead of my classmates. As importantly, Kenan was a role model for the pursuit of excellence and advancement through education and hard work. Kenan was the first of my family to attend college, specifically the Turkish Military Academy. Throughout his career, he was highly respected by his men and his colleagues for his fairness, hard work, and determination. He eventually rose to the rank of a brigadier general in the Turkish Armed Forces.

Career Decisions

I was the top student in my class throughout my primary education in Savur and my secondary education in Mardin.

Figure 33. The Abdulgani and Meryem family in 1971. Aziz was abroad studying and is not in the photo. From back left to right: Kazım, Yıldız, Edibe, Meryem, Kenan, Abdulgani, Yasemin, Tahir (uncle), Nezihe. Middle: Hasan, Seyran, Nurhan, Orhan, Zeynel, Sevim, Belma. Front: nieces and nephews.

Figure 34. Aziz in 3rd grade with big brother Kenan and sister-in-law Nezihe (1955).

Figure 35. Picture of the Mardin Lisesi high school soccer team in 1962. Aziz is the goalkeeper (top row, 2nd from right).

My favorite classes were Math, Turkish, French and Chemistry. In 10th grade an excellent Chemistry teacher inspired me to become a chemist. However, academics were not my only love. Like every boy throughout most of the world, I grew up playing soccer. In high school, I played goalkeeper for my high school (Mardin Lisesi), for Savur Spor (Savur), and for Mezopotamya Spor (Mardin) (Figure 35). I was very good because I had fast reflexes and was fearless. More than once my teammates carried me off the field on their shoulders because I had made critical saves that helped win the game. During this period, I was asked by the Turkish Soccer Federation to participate in regional trials for the Turkish Under-18 team. Although playing for the Turkish National Team had long been a dream of mine, I chose not to participate in the trials because I thought that my height and weight were not sufficient for a national caliber player. Even though I quit playing soccer after the 10th grade, my love of the game remains, and I am an ardent supporter of Turkish and American National Teams, Galatasaray Professional Turkish soccer team, and the University of North Carolina – Chapel Hill Women's Soccer Team.

When I graduated from high school I took the entrance exam for the B.Sc. Program in Chemistry at Istanbul University and, at the suggestion of five of my friends from Mardin who were interested in becoming physicians, also took the Medical School entrance exam. I did well on both exams, but my friends prevailed on me to join them in Istanbul University School of Medicine instead of continuing in chemistry. I began Medical School in November 1963 (Figure 36).

Figure 36. Aziz with his medical school histology class in 1964. Aziz is in the center next to his professor.

Medical School

Coming to a cosmopolitan city like Istanbul had both advantages and disadvantages. I made friends with Turks of different ethnic backgrounds including Alevi, Armenian, Jewish, Greek, Kurdish, as well as the descendants of Turkish refugees from all of the Balkan Countries. This enlightened my world view, especially with regard to the horrific effects of the Balkan Wars and World War I and the evil effects of religious and ethnic bigotry. Several of my Professors, most of them Jewish, had fled Germany and nearby countries before or during World War II; despite the fact that many of them were leaders in their fields, they were rejected by many western countries but were recruited to Turkish Universities where they contributed to raising the education there to European standards. The Turkish nation owes a great debt of gratitude to these outstanding professors for their contributions to our science, education and even linguistics.

The main disadvantage of attending the top medical school in Turkey, was my fear of failure. Despite finishing at the top of my high school class in Mardin, I was now in class with fellow students who had graduated from some of the best public and private high schools in Turkey. I was determined to show my classmates that a student from the "backward" southeast could succeed and even surpass students from more cosmopolitan areas. I decided that I could realize this goal by totally immersing myself in my studies to the exclusion of all else. I never went to a movie theater, concert, or a play in Istanbul. My only diversion during that time was my involvement in the Turkish Nationalist Movement, which was opposing the Communist/Internationalist movement that was gaining strength in the country. I never participated in physical violence but strongly believed that the "comrades" who occupied the main administrative building of Istanbul University and hung the hammer-and-sickle red flag on top of the building were wrong; I still believe that communism, as it is practiced, is evil.

In my second year of medical school, I learned for the first time about the DNA double helix; I was fascinated and decided to become a biochemist when I graduated. My first thought was to begin research training as soon as possible, so in my final year of medical school I consulted the Chair of the Biochemistry Department, Mutahhar Yenson, about the possibility of joining the department upon graduation. He expressed the opinion that anyone obtaining a medical degree, should practice medicine for at least two years before specializing in basic science research. So after graduation, at the top of my class, I returned to Savur to practice medicine in June of 1969.

Medical Practice

For the first six months after I returned to Savur, I turned a room in my family's house into a free clinic. Fortuitously, in the Fall of that year the Turkish Minister of Health passed through Savur, learned of my clinic, and suggested that I work for the Ministry of Health. Eventually, I was appointed Chief Medical Officer to a nearby village called Surgucu and was provided with a Jeep and a chauffeur. For the next year, I served people in Surgucu, in nearby villages and hamlets, and in a number of very remote villages. I was the first doctor that many of my patients had ever seen. I spent much of the salary I was paid by the Ministry of Health to buy drugs for my patients and toys for the small children whose families could not afford them. With simple medical procedures, I believe I saved the lives of many children.

One of the most challenging aspects of my medical practice, was that some of my female patients spoke only Kurdish; during that period and in that part of Turkey, families did not send their daughters to school, so they did not learn Turkish. Local translators were usually men, and thus the women were often uncomfortable explaining intimate health problems to a man from their village. I tried to circumvent the problem by learning Kurdish, but I never became fluent. Nevertheless, I think the women appreciated the effort; they often kept the prescriptions I had written as a talisman after using the drugs I prescribed.

Looking back, I remember the 18 months that I practiced medicine as the happiest time in my life. However, I also found the practice of medicine intellectually frustrating; for example, I wanted to understand why streptomycin killed the tuberculosis bacterium, but penicillin did not. So throughout the time I practiced medicine, I also applied for fellowships to study biochemistry abroad.

Ph.D. Studies: Cloning the Photolyase Gene

In 1971, I won a NATO fellowship to fund Ph.D. research in one of the member countries. I chose the United States, because it was the leader in scientific research in the world. I was admitted to the Department of Biochemistry Graduate Program at Johns Hopkins University, and entered there in 1971. I was totally unprepared for the problems I would encounter there. Although, I had taken English classes during my final year of medical school, I could not communicate with my professors and fellow students. In addition, because of my previous academic success and patriotic upbringing, I was self-assured and confident to the point of arrogance, and people avoided me. It was like being in solitary confinement. As a result, I left Johns Hopkins in June of 1972 and returned to Savur to regroup. After practicing medicine again for about 6 months, and a brief detour to England, I returned to the United States more mature and reasonably proficient in English, and applied to Dr. Claud S. Rupert at the University of Texas at Dallas (UTD). I was accepted into the UTD Biology Program there in 1973 and joined Dr. Rupert's lab in 1974 (Figure 37).

Figure 37. Aziz with his PhD mentor Claud (Stan) Rupert in 2009.

Dr. Rupert is the scientist who discovered the enzyme photolyase; this discovery, in 1958, marks the beginning of the scientific field of DNA repair. In the bacterium *E. coli*, exposure to UV light kills the organism; however subsequent exposure to visible light reverses the killing effect. This is called photoreactivation, carried out by the enzyme photolyase. When I joined Dr. Rupert's lab, the most outstanding question was "how does the enzyme absorb light". To answer this question it was necessary to have the enzyme in large quantities and high purity, but no one had been able to purify it in sufficient amounts. About the time I joined the Rupert lab, molecular cloning was invented at Stanford University. I immediately saw the potential of this approach for solving the photolyase production problem. I would clone the *E. coli* photolyase gene, amplify the enzyme, and then purify it and characterize its chromophores and action mechanism.

The first step, was to isolate a mutant defective in the photolyase gene so that I could use this mutant as a host for cloning. I devised a counter-intuitive experimental scheme to generate and select the mutant and performed the screen 1–2 times daily for 6 months before obtaining the first *phr* mutant. Along the way my self-confidence was challenged, not only by the difficulty of obtaining a mutant but also, during the period of repeated failures, by the comments of a labmate who told me that I did not have talent for lab research and should return to medical practice. The ultimate success of this experiment played a pivotal role in my evolution as a scientist because it required me to gather information from unrelated fields to create a method and because I persevered until the method worked. I believe, that there are three characteristics essential for a successful scientist: creativity based upon knowledge, hard work, and perseverance in the face of failure. Although the paper describing this method has only been cited 6 times (including two self-citations), for me it is one of my most important papers because it gave me the confidence to carry on research and equally it helped convince Dr. Rupert that I was a good student so that he gave me the freedom to pursue my research goals.

Using the mutant I had isolated, I cloned the *phr* gene of *E. coli* in 1975, and began experiments to characterize the plasmid carrying the gene. However, in 1976 I was called back to Turkey to fulfill my military service obligation. I returned to Texas four months later with the rank of Second Lieutenant

and resumed my work using the cloned gene to purify the enzyme. However, cloning a gene was such a major achievement at the time (I believe that *phr* was the first gene cloned east of the Rocky Mountains) that Dr. Rupert decided I had accomplished enough to earn a Ph.D. I started writing my doctoral dissertation in the Spring of 1977 and, with the encouragement of Dr. Rupert, applied to three leading DNA repair labs. I did not receive an offer from any of them, probably because I had not published. I had been so engrossed in doing experiments that I had not taken the time to write up the 6–7 papers I had material for. Moreover, gene cloning was new and its utility was not appreciated yet by many in the field. Fortunately, I learned from a fellow graduate student that Dr. W. Dean Rupp of Yale University was planning on cloning the *uvrA*, *uvrB and uvrC* genes responsible for nucleotide excision repair in *E. coli.* I applied to Dr. Rupp and, based upon Dr. Rupert's strong personal recommendation, Dr. Rupp offered me a position in his lab. I defended in July of 1977 and left UTD in September to join Dr. Rupp's lab, still not knowing how photolyase absorbs light.

Post-Doctoral Work: Maxicells and Dual Incision I

When I joined the lab of Dr. Rupp, Yale University was one of the top three DNA research centers in the world and an exciting research environment. In addition to Dean Rupp, other pioneers in the field of repair and recombination were there, including Paul Howard-Flanders, Charles M. Radding and Fred Hutchinson. I cloned the *uvrA*, *uvrB* and *uvrC* genes in quick succession. While at Dallas, I had begun working on a method, which I called Maxicells, to identify the proteins encoded by cloned genes. At Yale, Dr. Rupp made suggestions to improve the method, which were crucial to its eventual success. It took almost a year to work out the details, but eventually the method worked. The paper describing Maxicells was published in 1979, and became an instant hit because it was applicable to identifying any plasmid-encoded protein. The method was widely used throughout the 1980s, and is to this day my most cited research paper.

Having cloned the *uvrA*, *uvrB* and *uvrC* genes, I used the Maxicell method with radioactive tracers to label and identify and purify the proteins encoded by these genes. Up to this point, the classical model for nucleotide excision repair was that a UV endonuclease incised the damaged strand 5′ to the damage and an exonuclease removed the damage in the 5′ to 3′ direction in the form of a 4–6 nucleotide fragment containing the damage. Much to my surprise, in the Spring of 1982 I found that when I reconstituted the incision reaction in vitro using purified proteins, the UvrABC nuclease made concerted dual incisions, one 7 nucleotides 5′ to the dimer and the other 3–4 nucleotides 3′ to the dimer, releasing a 12–13 nucleotide long fragment carrying the dimer. I named the enzyme "ABC excinuclease" to emphasize the unique dual incision mechanism. This was a major discovery in the field of DNA repair; however, because there were several other groups working hard on the same question, I could not tell anyone except a few lab colleagues about this result until we were ready to present it at a meeting and to publish it. Dr. Rupp presented the result for the first time at an international meeting on recombination and repair in France in the Spring of 1982. I still run into colleagues, who say that this talk generated huge excitement at the meeting. Dr. Rupp's talk was published in the meeting proceedings, and a full paper describing my work was published in 1983.

While I was in Dr. Rupp's lab other exciting events were also happening in my personal life. Back in Texas I had become a close friend of Gwen Boles, a graduate student in the same department at UTD. Gwen graduated three months before me and took a post-doctoral position in New York, working on the molecular basis of thalassemia. We continued to see each other on weekends when I moved to Yale and married in 1978 (Figures 38 and 39). However, it was another 2 years before Gwen completed her work in New York, moved to Yale, and joined Dean Rupp's lab to work on regulation of DNA repair genes in *E. coli*. Although living apart was not ideal, the additional time that Gwen spent in New York allowed her to eventually publish 5 papers from her post-doctoral work there.

In 1981, encouraged by my research successes, I began applying for faculty positions. I applied to about 50 universities and was turned down by all of them, some without even a reply to my application. Then I received a call from Mary Ellen Jones, the Chair of the Department of Biochemistry at

Figure 38. Picture of Aziz and Gwen in 1994 in Chapel Hill, NC.

Figure 39. Aziz with Gwen and Rose in front of Ataturk Mausoleum, December 15, 2015.

the University of North Carolina at Chapel Hill. Dr. Jones was interested in recruiting molecular biologists to modernize the department. Gwen and I visited Chapel Hill, and we were both offered faculty positions in the Spring of 1981. Because I was working on the reconstitution of ABC excinuclease and felt that I could not take a 6-month break to set up a new lab, we accepted the positions on the condition that we could defer moving for a year. Dr. Jones agreed, and that enabled me to submit the paper describing the reconstitution of ABC excinuclease and the dual incision mechanism in the fall of 1982, just before moving to Chapel Hill. This also allowed Gwen and me to write our first NIH grant proposal to work on photolyase. The proposal was funded, and as a result when we arrived in Chapel Hill most of our equipment was already in place, and we were able to start experiments 3 days after we arrived.

Photolyase: "As Complete as Any Research Study Can Be"

When I started my own lab at UNC-CH, I decided to resume working on photolyase, specifically on identifying the chromophore and solving the action mechanism. In a relatively short period of time, we overexpressed and purified the enzyme and discovered that the enzyme has not one, but two cofactors, $FADH^-$ and MTHF that absorb light. In a series of experiments with collaborators from around the world, we found that MTHF acts as an antenna which absorbs light energy and transfers that energy to the FAD cofactor which carries out catalysis. Over the next 30 years, we and our collaborators defined the molecular mechanism in great detail and have traced all of the steps of the repair reaction in real time, from light absorbance to splitting of the dimer and return of the electron to the flavin cofactor. My work on photolyase has, with interruptions, spanned over 40 years and involved collaboration with numerous colleagues who were leaders in cofactor chemistry, flavin photochemistry, crystallography and ultrafast chemistry. It was therefore gratifying when a colleague recently wrote in a commentary on a paper we published in 2011 with our collaborator Dongping Zhong that "with this paper the story of PL (photolyase), originating 62 years ago, has come to be as complete as any research subject can be".[179]

Transcription-Coupled Repair; Yunus Emre Destani

In 1985 and 1987, Philip Hanawalt and colleagues reported that transcription strongly stimulates nucleotide excision repair in human cells and in *E. coli*. They suggested that RNA polymerase stalled at a damage site increased the rate of damage recognition, which is the rate-limiting step in excision repair. We tested this model in vitro using purified *E. coli* proteins and found that RNA polymerase stalled at damage actually inhibited repair. From this we proposed that an additional factor recognized stalled RNA polymerase, displaced it from the damaged site, and simultaneously facilitated assembly of the excision nuclease at the damage. We identified and purified such a factor which we named TRCF (transcription-repair coupling factor). We went on to show that TRCF is the product of the *mfd* gene first described by Evelyn Witkin in 1956, and that purified TRCF, RNA polymerase, and ABC excision nuclease are sufficient to reconstitute transcription-coupled repair in vitro. I consider the paper describing this work to be our most aesthetically pleasing, both scientifically and stylistically. We made a hypothesis, obtained the necessary reagents to test it, and found the hypothesis to be correct. In the process we solved a mystery of 25 years standing (mutation frequency decline). The paper is well-written, states the problem concisely, and proceeds to describe the experimental results succinctly. The data is clear and unambiguous and the model has stood the test of time. To my Turkish colleagues who inquire about my research, this is my Yunus Emre Destani (Yunus Emre Opus), because Yunus Emre, a mystic poet who lived in the 14th century, is to the Turkish language what Chaucer is to the English language, and every Turk aspires to the perfection Yunus Emre achieved in his chosen field.

Excision Repair in Humans, Dual Incision II ("Known Only to God and Me"), and Molecule of the Year

In 1987, I turned my attention to the mechanism of human nucleotide excision repair which had remained poorly understood for over 20 years. We decided to pursue a biochemical approach to understand the reaction mechanism, and focused initially on what we viewed as the most important question: do human cells utilize a UV endonuclease/exonuclease for excision or is there a dual incision mechanism similar to the one we had found in *E. coli*? For five years, we tried many assay systems, cell types, different cell extract preparations, and different types of substrates, to no avail. Finally on November 8, 1991 we captured the excised oligonucleotide: it was a 27-mer ("nominal 30-mer") released by dual incisions. Yes, the mechanism was by dual incisions, but the dual incisions were different than in *E. coli*. This discovery was one of the highlights of my research career. When I first saw the 27-mer, I told Gwen "there is an important biological fact about humans that is known only to God and me". We followed up on this discovery, by isolating and purifying all of the proteins necessary for the dual incision reaction, and reconstituting the reaction in vitro from completely purified components. This work, combined with our elucidation of the mechanism of TCR played an important role in the selection of DNA Repair as "Molecule of the Year" by *Science* Magazine in 1994. For this issue, I, Paul Modrich and Philip Hanawalt were asked to summarize the exciting discoveries in the field of DNA repair by our respective laboratories, as well as those of a dozen other laboratories in the preceding year.

Repair Map of the Human Genome; Piri Reis Map

After the discovery of dual incisions in humans, we wanted to know the fate of the excised oligomer in human cells, but were unable to isolate the 30-mer from UV-irradiated human cells. After spending 20 years characterizing

human excision repair in vitro, we finally captured the 30-mer produced in vivo. This has allowed us to map the sites of repair across the entire human genome at single nucleotide resolution. This repair map shows, in a geographic sense, repair mountains, valleys and canyons corresponding to regions of high, average, and low or no repair. This method will likely help us understand factors other than the primary repair proteins that affect repair efficiency and may have applications to improve chemotherapy. Personally, this is the most satisfying accomplishment in my lab in the last decade, and to Turkish colleagues I refer to it as "My Piri Reis Map". Piri Reis was a Turkish admiral and cartographer who drew the world map in 1513 with a level of accuracy unrivaled by any other cartographer of his period. He is revered by Turks as a great scientist, arguably the last great scientist of the golden age of so-called "Islamic Science". After submitting the paper describing this result, I went on a lecture tour of Peru and told Gwen that "if my plane hits the Andes and I die, I will die a happy man."

DNA Damage Checkpoints

Cells respond to DNA damage by repairing it, by activating signal transduction pathways for arresting cell cycle progression, by changing the transcription profile, and by inducing apoptosis. These responses are important for cellular homeostasis and have been the subjects of detailed studies by many investigators. However, because of the very nature of the phenomena investigated, the biochemical analyses of these processes, with the exception of apoptosis, have been limited. With this general view, we decided to apply our experience in DNA repair to investigate the biochemistry of checkpoint activation. For the past 15 years, we have made significant contributions to the biochemistry of DNA damage checkpoint activated by UV damage. We developed several in vitro systems that captured specific steps in the signaling pathway. Perhaps, our most physiologically relevant accomplishment, has been the coupling of human nucleotide excision repair with the DNA damage checkpoint response in a completely defined system. I look at this work as the ultimate in reductionist biochemical research that aims to explain complex cellular phenomenon in a minimalist in vitro system.

Cryptochrome and the Circadian Clock

Photolyase is not universally distributed in the biological world, and its presence in humans had been controversial for 35 years after its discovery in bacteria in 1958. In 1993 we conducted an exhaustive study on this subject and published a paper stating categorically that humans do not have photolyase. This result applied to both the classic photolyase that repairs cyclobutane-type pyrimidine dimers and another type of photolyase discovered by T. Todo that repairs (6–4) pyrimidine–pyrimidone adducts. Then in 1995, Human Genome Sciences released the sequences for a number of partial human cDNAs, and among these was listed a photolyase homolog. We immediately obtained the cDNA for the entire gene, and shortly thereafter, discovered a second gene with high sequence similarity to photolyase. After cloning and expressing both genes, we found that neither of the recombinant proteins nor cells expressing the proteins had detectable photolyase activity of either type. We were still trying to decide what to do with these results, when "chance favored the prepared mind".

In May of 1996, returning from a visit to Turkey, I read an article about the circadian clock and jetlag by Dr. William Schwartz in a flight magazine. I was most intrigued by the setting of the clock by blue light (wavelengths similar to those absorbed by the photolyase chromophores) and the fact that in some blind mice and people who lack conscious light perception, the circadian clock still responds to light because the "circadian visual system" is anatomically and physiologically distinct from the image-forming visual system. After reading this article I thought perhaps the human photolyase paralogs we had found might in fact be clock proteins that sense blue light. I discussed this with my lab and suggested that we call these proteins cryptochromes 1 and 2 (CRY1 and CRY2) in analogy with the plant blue light photoreceptors which also had sequence similarity to photolyase. The paper describing this work was published in *Biochemistry* in November of 1996, and it appears that it escaped the attention of the entire circadian clock community.

To test this claim I immediately set out to learn as much as I could about the circadian clock, and neuroscience. By the end of 1997, we had shown that cryptochromes were highly expressed in the two anatomical locations critical to the clock, namely the ganglion cell layer of the retina, and the suprachiasmatic nucleus (SCN) in the brain which is the neurological center of the clock in mouse and man. In particular *CRY1* mRNA exhibited high amplitude daily rhythmicity in the SCN. This was sufficient circumstantial evidence for us to publish a paper in *PNAS* claiming that the mammalian CRYs are circadian photoreceptors. This paper electrified the circadian clock community, but still we needed evidence of causality. To prove our contention we had to show that mutations in the *CRY* genes altered the clock. We constructed a CRY2 mutant, and when it was tested in the laboratory of our collaborator Joseph Takahashi, it was apparent that even though the mutation affected sensitivity of the clock to light, even in complete darkness, it had an effect on the clock. We concluded, that CRY2 had both light-dependent and light-independent effects on the clock. In the meantime, our first paper on the potential role of CRY in the circadian clock led to the identification of a CRY homolog in *Drosophila,* and a *Drosophila* CRY mutant with greatly reduced photosensitivity was also isolated. Our work, also led to re-evaluation of *Arabidopsis* CRY mutants, and experiments performed by plant biologists showed that CRY also plays a role in the *Arabidopsis* circadian clock. Our CRY2 mutant mouse paper and the *Drosophila* and *Arabidopsis* papers were published within a week of one another. This, along with other important progress being made in the field, led to the circadian clock as runner-up for *Science* Magazine "Molecule of the Year" in 1998.

Later in 1999, our group, in collaboration with T. Todo and J. S. Takahashi and another group of Dutch and Japanese colleagues, made mouse mutants defective in both CRYs and found that they no longer had a functioning circadian clock. There was rapid progress in the field, and by 2000 there was a reasonably detailed model for the clock in which CRY plays the role of the primary transcriptional repressor in the clock circuitry generated by a transcriptional and translational feedback loops. Current evidence indicated, that CRY is primarily, if not exclusively, a repressor in mammals with no photoreceptor function, while in *Drosophila* it is the primary circadian photoreceptor. The discovery of cryptochrome as a circadian protein has given me a profound sense of gratitude and personal satisfaction for providing me the opportunity to contribute to an entirely different field of research from DNA repair and thus interacting with a new set of colleagues and a new way of thinking.

Full Circle

For the past 20 years, we have been working on the mechanism by which CRY participates in the circadian clock in mammals and its photoreceptor function in *Drosophila* and have contributed to the current clock models for both organisms. Our work has also led us to discover, that the circadian clock regulates excision repair in mice and that the carcinogenesis of UV light exhibits a circadian pattern. We are currently analyzing the circadian effect of repair in humans and the potential applications of this knowledge to chemotherapy regimens. Figure 40 shows a picture of our current lab members.

Figure 40. A recent photo of the Sancar lab (September 2015). From front right: Laura Lindsey-Boltz, Fazile Canturk, Aziz Sancar, Alana Oktay. From back right: Christopher Selby, Yanyan Yang, Yi-Ying Chiou, Jinchuan Hu, Michael Kemp, Muhammet Karaman, Wentao Li, Sheera Adar, Gulnihal Erkmen, Hiroaki Kawara.

Concluding remarks

I have had the good fortune of having parents who instilled a strong work ethic in me and the value of learning. I have been fortunate to have had excellent teachers throughout my education from primary school in Savur through high school in Mardin, and Medical School in Istanbul, and excellent mentors in graduate school and post-doctoral work in Texas and New Haven. I thank my family for their love. I am grateful to my wife, Gwen, for her love and support—or in the words of one of my mentors, "Aziz, I don't think you would have survived without Gwen". I thank my goddaughter, Rose Peifer, who has added joy to my life and makes me feel young. Finally, I thank Gwen and Rose for keeping me on the straight and narrow.

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 8502–8527
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Received: February 12, 2016
Published online: June 23, 2016

