2015 Nobel Prize in Chemistry for DNA repair

The Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich and Aziz Sancar 'for mechanistic studies of DNA repair'[1]. These three scientists have studied cellular functions extensively and have suggested how the knowledge gained from these studies can be used, for example, in the development of new cancer therapeutics. Tomas Lindahl (Figure 1) was born in Kungsholmen, Stockholm, Sweden in 1938. He received a Ph D degree in 1967 and an MD degree in 1970 from the Karolinska Institutet in Stockholm. Lindahl carried out his postdoctoral research at Princeton University and Rockefeller University. During 1978–1982, he worked as a professor of medical chemistry at the University of Gothenburg in Gothenburg, Sweden. He is currently associated with the Francis Crick Institute and Clare Hall Laboratory, Hertfordshire, United Kingdom. He contributed significantly to the understanding of instability, decay and repair of DNA at the molecular level in bacterial and mammalian cells[2].

Aziz Sancar (Figure 1, born in 1946) is currently the Sarah Graham Kenan Professor of Biochemistry and Biophysics at the University of North Carolina at Chapel Hill, NC, USA. He received his MD degree from Istanbul University, Turkey and his Ph D degree in 1977 from the University of Texas at Dallas, USA. The research at Sancar's laboratory involves mammalian DNA excision repair, mammalian DNA damage checkpoints and mammalian circadian clock[3]. Paul Modrich (Figure 1, born in 1946) is the James B. Duke Professor of Biochemistry at Duke University, a member of the Duke Cancer Institute and also an investigator at the Howard Hughes Medical Institute, USA. He received an undergraduate degree in 1968 from Massachusetts Institute of Technology (MIT) and a Ph D degree in 1973 from Stanford University. Modrich's work primarily involves strand-directed mismatch repair. He showed how DNA mismatch repair is used as a copy editor to prevent errors from DNA polymerase[4].

DNA damage

DNA damage generally refers to a change in the chemical structure of DNA, which includes DNA strand break (single or double), loss of base from DNA, or a chemically modified base. Damages to DNA can occur via two major pathways: endogenous cellular processes and reactions mediated by exogenous agents[5]. Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, singlet oxygen, hydroxide ion, peroxynitrite, etc. contribute significantly to DNA damage. Although most of the ROS are produced as by-products of the normal metabolism of oxygen, ROS can also be generated by exogenous sources such as ionizing radiation. ROS can cause DNA single-strand break or double-strand break (Figure 2 a). The base guanosine in the deoxyguanosine moiety of DNA is a preferred target for oxidative damage as this

modification produces 8-oxo-dG (Figure 2 b), which is a major biomarker of oxidative stress. The amount of 8-oxo-dG in both mitochondrial DNA and nuclear DNA of mammalian tissues increases with age. The other modifications include hydrolytic attack and uncontrolled alkylation. While the hydrolysis causes deamination, depurination and depyrimidination, the alkylation (usually methylation, Figure 2 b) leads to the formation of 7-methyl deoxyguanosine, 1-methyl deoxyadenosine and 6-O-methyl deoxyguanosine. The spontaneous depurination may account for the loss of up to 10000 guanine and adenine bases every day[2]. Furthermore, the spontaneous deamination of cytosine to uracil occurs at a rate of 100 bases per cell per day. The 2015 Nobel Prize in Chemistry

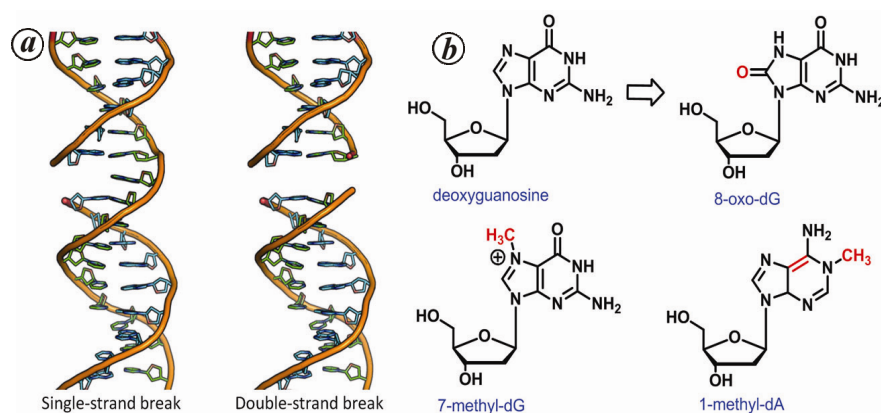**Figure 1.** Photographs of Tomas Lindahl, Aziz Sancar and Paul Modrich.

Figure 2. *a*, Single- and double-strand break in DNA (image: www.bioquicknews.com). *b*, Oxidative modification of deoxyguanosine (dG) produces 8-oxo-dG and methylation of deoxyguanosine and deoxyadenosine produces 7-methyl dG and 1-methyl dA respectively.

was awarded for the discovery of the processes that repair the above-mentioned damages in cells[1].

Tomas Lindahl: Base excision repair mechanism

The main interest of Lindahl was to understand fundamental DNA repair mechanisms related to cancer therapy and human genetic disorders[6]. His major achievement was the characterization and quantification of spontaneous, endogenous DNA damage during the 1970s and 1980s. During the initial period, he studied the instability of DNA and measured DNA decay including the rate of base loss. On the basis of extensive studies, he suggested that the hydrolytic depurination (Figure 3 a), deamination of cytosine residues (Figure 3 b), oxidation of guanine and pyrimidine residues and methylation of adenine residues amount to 10,000 potentially mutagenic and cytotoxic changes per day in a human genome[2]. As the abasic sites are non-coding and they block replication if not repaired, Lindahl believed that special DNA repair enzymes and mechanisms must exist to counteract the damages caused by endogenous processes. Subsequently, he became interested in understanding DNA damage and the fundamental DNA repair mechanisms at molecular level. An excellent review of his work on DNA damage caused by endogenous factors and the DNA mechanism was published in *Nature*[2].

Lindahl discovered the base excision repair (BER) mechanism as the major cellular defense against DNA damage caused by endogenous processes[6]. He discovered several key DNA repair enzymes/proteins and demonstrated their mode of action. He was able to reconstitute two variants of BER (short- and long-patch BER) with purified proteins and showed that many DNA repair enzymes such as DNA glycosylases that cleave the sugar-base bonds (e.g. uracil-DNA glycosylase), 3-methyladenine-DNA glycosylase and DNA glycosylase that mediates the release of oxidized base from DNA, AP endonucleases responsible for incision of double-stranded DNA, the O6-methylguanine (O6-MG)-DNA-methyltransferase (MGMT, also known as AGT, or AGAT), DNA dioxygenases are involved in DNA repair mechanisms. It should be noted that MGMT transfers

a methyl group from methylated DNA to one of its cysteine residues to form a methylated cysteine within the protein molecule. Interestingly, this led to the first demonstration of activation of a transcription factor by a post-translational modification event[6].

A general mechanism of DNA repair involving the BER is shown in Figure 4. According to this pathway, DNA glycosylases initially recognize the lesion in DNA. These enzymes bind to the DNA, flip the damaged base out of the double helix, and finally cleave the N-glycosidic bond, leading to the formation of an apurinic/apyrimidinic (AP) site. The endonucleases (e.g. AP-1 and AP-2 in human) then cleave an AP site to produce a 3′-hydroxyl adjacent to a 5′-deoxyribose moiety. The resulting fragments with a hydroxyl on its 3′-end and a phosphate on its 5′-end are ligated. In humans, during the short-patch BER,

DNA ligase III and its cofactor XRCC-1 form a protein complex, which catalyses the nick-sealing step. Finally, the DNA polymerases (e.g. poly β) create DNA molecules by assembling nucleotides. Lindahl identified DNA ligases in eukaryotic cells and found that these enzymes use ATP rather than NAD as cofactor. The main DNA ligase that functions in mammalian cells during BER mechanism has been identified as DNA ligase III.

Aziz Sancar: Nucleotide excision repair mechanism

Sancar discovered the process by which cells repair UV damage by removing an entire nucleotide, instead of the base in a damaged DNA[3]. He demonstrated that the excinuclease cuts out the damaged part of DNA, taking a piece about 12
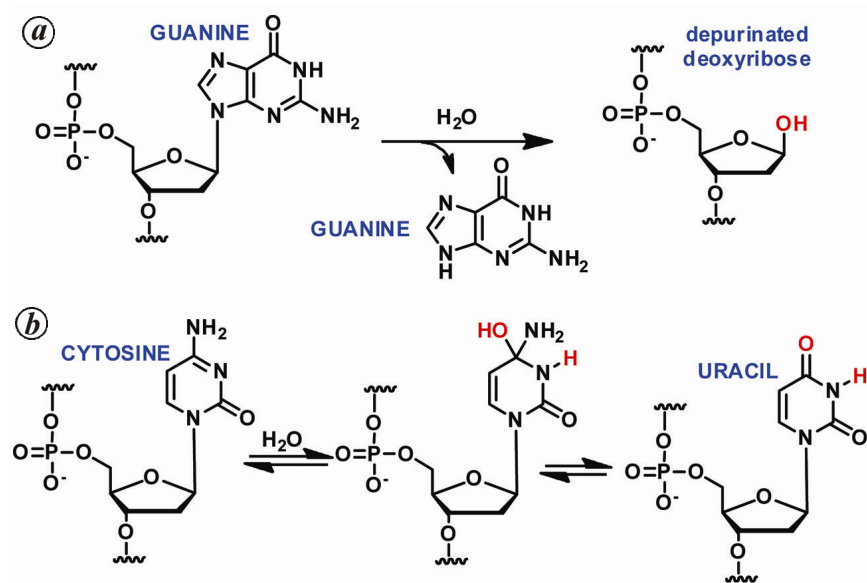

Figure 3. *a*, Spontaneous depurination involving removal of guanine base to produce depurinated deoxyribose. *b*, The conversion of cytosine base to uracil in DNA by deamination.
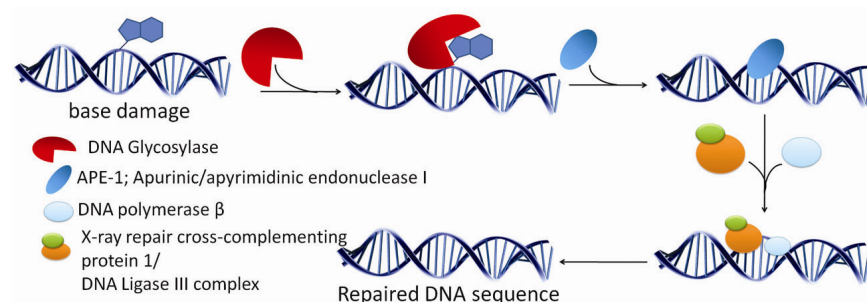

Figure 4. DNA repair involving base excision repair mechanism.

nucleotides long in total. This is followed by the action of DNA polymerase, which fills in the gap. Finally, the DNA ligases stitch the pieces back together to get repaired DNA. Although, in bacteria, the photolyase enzymes that use light mediate the repair of UV-induced DNA damage, Sancar found that nucleoside excision repair (NER) pathway exists in mammalian cells, which efficiently repair the damaged DNA in dark. One of the major damages to the DNA by radiation is the formation of pyrimidine dimers. These dimers are formed from thymine (T) or cytosine (C) bases via photochemical reactions (Figure 5 *a*). The photo-induced dimerization is known to alter the basic structure of DNA and consequently inhibit polymerase, which stops the replication process. The dimers generated by irradiation are generally repaired by several proteins by NER pathway, but unrepaired DNA leads to melanomas (skin cancer) in humans (Figure 5 *b*).

The major steps of NER involve (i) the recognition of the damaged nucleotide, (ii) dual incisions placed around the lesion, (iii) cleavage of the oligomer and its release, (iv) synthesis of DNA strand to fill in the gap and (v) ligation mediated by ligases[3]. In humans, NER is carried out by six repair factors (RPA, XPA, XPC, TFIIH, XPG and XPF•ERCC1) (indicated as multiple protein complexes in Figure 6). The recognition factors for the human excision nuclease are RPA, XPA and XPC and each of these factors is a DNA-binding protein with some preference for damaged DNA. With the help of 3′- and 5′-excision nucleases XPG and XPF/ERCC1 respectively, an incision is placed 6 ± 3 and 20 ± 5 nucleotides upstream and downstream of the dimer, leading to the removal of ~24–32 nt single stranded DNA segment (eukaryotes) including the lesion. The gap generated by the excision is filled in using information from the complementary strand with the help of a polymerase (e.g. pol β). The continuity of the DNA is finally restored by ligation with DNA ligase I to obtain a repaired DNA sequence (Figure 6). As the complementary strand is important for the generation of new strand, complex lesions such as pyridine dimer formation combined with double strand break are difficult to repair.

Paul Modrich: Mismatch DNA repair mechanism

Modrich discovered a repair mechanism in cells that corrects wrongly included base pairs in DNA, which is known as DNA mismatch repair[7]. He first identified the mismatch repair pathway in bacteria. Interestingly, the bacteria were able to repair the mismatches introduced into the DNA of viruses infecting them. Following these initial studies, Modrich recreated and studied the mismatch repair process *in vitro* and suggested that the mismatch repair may correct 99.9% of such errors in human DNA during replication. His discovery was an important milestone in DNA research related to human diseases. For example, it is known that congenital defects in mismatch repair can cause a hereditary variant of colon cancer.

For several years, Modrich's research was focused on the understanding of how cells repair mismatched bases in DNA. In a series of studies, he showed that bacteria use dam methylase to mark the damaged DNA with methyl groups, which guide a restriction enzyme to cleave the DNA at the right place[7]. In 1989, he reported a system consisting of DNA polymerase III, exonuclease I and DNA ligase that could repair DNA mismatches *in vitro*. In this case, the mismatch repair was guided by DNA methylation. As shown in Figure 7, the mismatch repair (MMR) proteins recognize the part of DNA having mismatched base-pairs that are produced during DNA replication, homologous recombination, spontaneous cytidine deamination or other forms of DNA damages discussed earlier. In the second step, two other proteins, MSH6 and MSH2, are involved in the recognition of mismatch (e.g. G–T base pair). It is known that the protein
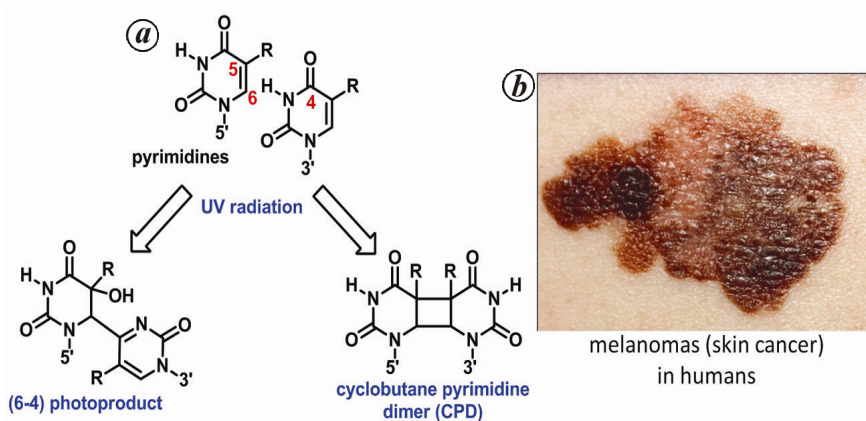

Figure 5. *a*, Radiation-induced dimer formation in pyrimidine bases. *b*, Melanomas (skin cancer) caused by unrepaired DNA in humans (Source: Wikipedia).
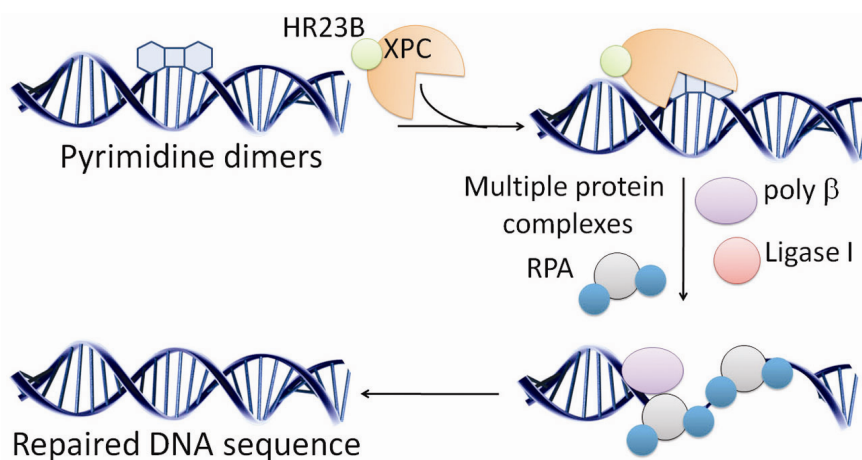

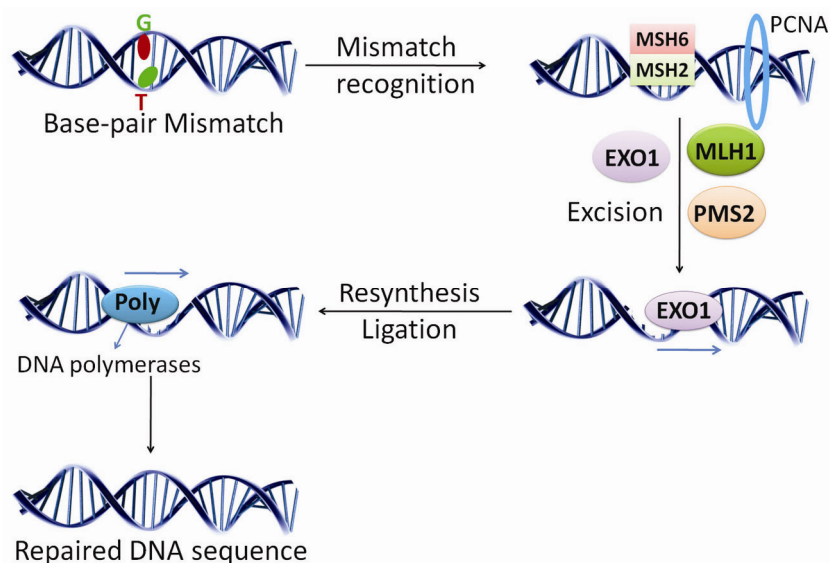**Figure 6.** Repair of DNA by nucleotide excision repair pathway.

Figure 7. Mechanism of DNA mismatch repair. The pathway involves mismatch recognition, excision, resynthesis and ligation of DNA strands.

hMSH6 in human combines with hMSH2 to form the active protein complex, hMutS alpha. Proliferating cell nuclear antigen (PCNA), acts as a cofactor for DNA polymerase δ in eukaryotic cells. The DNA clamp, PCNA, achieves its processivity by encircling the DNA as shown in Figure 7 and acts as a scaffold to recruit proteins that are important for DNA replication and DNA repair. The exonuclease 1 (EXO1) also interacts with MSH2 to facilitate the nucleotide removal. It should be noted that MLH1 is a human homolog of the *E. coli* DNA mismatch repair gene, *mutL*, which is known to mediate interactions between proteins during mismatch recognition and discrimination and removal of DNA strands. As an endonuclease, PMS2 then introduces nicks into a discontinuous DNA strand. Further processes involving resynthesis, ligation and action by DNA polymerases produce the DNA sequences with correct base pairs.

Summary

In summary, the research findings of the three scientists, Tomas Lindahl, Paul Modrich and Aziz Sancar, completely changed the way we understood the stability, damage and repair of DNA. These findings also became essential for future research on how the defects in DNA repair mechanisms could be used to combat several diseases, including cancer, metabolic and neurological disorders. Recent evidences suggest that the repair systems do not work in many forms of cancer, and therefore, the inhibition of remaining repair processes may help to stop the growth of cancer. For example, the FDA-approved drug for cancer, *olaparib*, blocks the tumour growth by inhibiting the poly ADP ribose polymerase (PARP), an enzyme involved in DNA repair. Therefore, the fundamental research carried out by the 2015 Nobel Laureates in Chemistry has not only helped us understand the DNA repair and mechanisms, but also laid the groundwork for the development of therapeutic drugs for life-threatening diseases such as cancer.

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