CBS 530 Assignment No 3

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Review of the paper: DNA Repair

Abstract

In this report the author gives her own view on the articles of Jkimball[1].

1 Introduction

DNA is a polymer which is strung together from monomers called **deoxyribonucleotides**. DNA is a crucial molecule in living cells and it has a fascinating structure which supports two most important functions of DNA:
(1) Coding for the production of protein and
(2) Self replication
So that an exact copy is passed to the offspring cells.

Each deoxyribonucleotide consists of three components:
1. A sugar
2. A phosphate group and
3. Nitrogenous base

The double helix of DNA has three features:
1. It contains two polynucleotide strands wound around each other.
2. The backbone of each consists of alternating deoxyribose and phosphate group. The phosphate group bonded to the $5'$ carbon atom of one deoxyribose is covalently bonded to the $3'$ carbon of the next. The DNA strands are assembled in the $5'$ to $3'$ direction.
3. The Purine(Adenine and Guanine) and Pyrimidine(Cytosine, Thymine and Uracil) attached to each deoxyribose. Each base forms hydrogen bonds with the one directly opposite it, forming base pairs.

2 Review

Cellular DNA is subjected to continual attack, both by reactive species inside cell and by environmental agents. DNA can be repaired by these attacks. How important is DNA repair:
1. The repository of hereditary information and

2. The blueprint for operation of individual cells.

DNA repair is involved in processes that minimize cell killing, mutations, replication errors, persistence of DNA damage. Abnormalities in these process have been implicated in cancer and aging. The repair of damaged DNA is possible because the damaged base cause a mismatch between bases that causes a defect in the double helix that can be recognized by enzymes. Three common steps in DNA repair:
1. Excision removal of the damaged nucleotide
Enzyme = Various nuclease
2. Re-synthesis replace the missing nucleotide
Enzyme = DNA polymerases
3. Ligation seals the nick in the sugar-phosphate backbone
Enzyme = DNA ligase

3 What are the agents that damage DNA

• Certain wavelengths λ(=v/f) of radiation. Three types of radiation are:
· Light
· Heat and
· Ionizing radiation.
◦ Ionizing radiation can penetrate cells and create ions in the cell contents. These can cause permanent alteration in DNA, that is mutations.
◦ The somewhat shorter Ultraviolet rays(260 nanometer)and Gamma and x-rays are electromagnetic(like light) that can be absorbed by DNA. Ultraviolet light is absorbed by the nucleic acid bases and the result influx of energy can induce chemical changes. Ultraviolet light introduces covalent bonds between adjacent Thymine or Cytosine bases in the same DNA strand.
◦ Highly-reactive oxygen radicals(oxidative changes) and biochemicals(methylation) produced during the absorption of oxygen and the release of energy and carbon dioxide by cells.
◦ Chemicals in the environment like in many hydrocarbons, in cigarette smoke, some plants and microbial products, e.g. moldy peanuts.
◦ Chemical used in the treatment of cancer disease.

4 Types of DNA damage

• All the four bases in DNA(A,T,G,C) can be covalently changes at various positions by
◦ Deamination spontaneous hydrolysis of an amino group, convert Cytosine to Uracil.

• Mismatches of the bases because of a failure of proofreading during DNA replication
◦ In place of the pyrimidine T incorporation of U which is only in RNA. A-T paring involves the formation of two H-bonds between the two nucleotide, while the C-G involves three H-bonds.
• Breaks in the backbone
◦ Could be on one strand that is a single-stranded break(SSB) or
◦ On both strands that is a double-stranded break(DSB) or
◦ By some chemicals
• Cross links covalent linkages between bases
◦ Some of drug used in cancer chemotherapy also damage DNA by alkylation.
◦ On the same or on the opposite DNA strand e.g.
(1) Phosphodiester linkage
(2) Nitrogenous base(A,T,G,C), $5'$-phosphate group of one nucleotide is joined with the $3'$-hydroxyl group of other.

5 Repairing Damaged Bases

Damaged or mismatched bases can be repaired by several mechanisms:

• Direct chemical reversal(DCR)
• Excision repair(ER) the damage can be accurately repaired by cutting it out(excision) and replacing it with new DNA synthesized using the complementary strand as template. Like

Excision repair is divided in three modes with specialized sets of enzymes.
◦ Base Excision Repair(BER)
◦ Nucleotide Excision Repair(NER)
◦ Mismatch Repair(MMR)

6 Direct Reversal of Base Damage

Direct reversal is the sealing of a subset of nick in DNA by DNA ligase. DNA ligase can only seal nicks having $5'$-phosphate and $3'$-hydroxyl. Direct reversal is repair of o^6-methyl guanine by transfer of the methyl group(CH_3) from the DNA to a cytosine in a protein and protein can only do it once, so the removal of each methyl group requires another molecule of protein that's why they are quite wasteful.
Some of the methyl group can be removed by a protein encoded by MGMTgene. (The DNA repair enzyme methyl-guanine-methyl-transferase(MGMT) is a protein that has a differential expression pattern in different cell types within the body).

Most of these changes are repaired by enzymes, called glycosylases, that remove the mismatched T to C. This can be changed without break the DNA backbone.
Cell needs general mechanism which can correct all sorts of chemical damage with a limited toolbox. This requirement is met by the mechanisms of excision repair.

7 Base Excision Repair(BER)

To remove incorrect bases(like uracil) or damaged bases(like 3-methyladenine). four steps are there:
◦ Removal of the incorrect base by an appropriate DNA N-glycosylase. 8 genes encoding different DNA glycosylase each enzyme responsible for identifying and removing a specific kind of base damage.
◦ Removal of deoxyribose phosphate in the backbone, producing a gap.
◦ Replacement with the correct nucleotide, this relies on DNA polymerase beta (is a eukaryotic DNA polymerase. Pol B is responsible for adding new nucleotide to a growing chain by catalyzing a nucleotidyl transfer reaction, it is primarily involve in DNA repair. Pol B is a single polypeptide chain of 335 amino acids).
◦ Ligation of the break in the strand.

8 Nucleotide Excision Repair(NER)

NER differs from BER in several ways.
• It uses different enzymes.
• There may be only a single incorrect base to correct, NER removes a large patch around the damage. The steps:
◦ The damage is recognized by one or more protein factors.
◦ Binding of a multi protein complex at the damaged site. TFIIH fulfills a dual role in transcription initiation(TI). In TI TFIIH is thought to be involved in unwinding of the promoter site and to allow promoter clearance. Subunits of Transcription Factor TFIIH also involved in NER.
◦ Cuts are made on both the $3'$ side and the $5'$ side of the damaged area so the system containing damage can be removed.
◦ Filling of the resulting gap by a DNA polymerase using opposite strand as a template.
◦ A DNA ligase or ligation binds the fresh piece into the backbone.

9 Xeroderma Pigmentosum(XP)

XP is caused by a defect in NER mechanism. XP is a inherited disease of humans which
• Pigmented lesions on areas of the skin exposed to the sun.
• Early onset of skin cancer at high incidence.
• Elevated frequency of other forms of cancer.

DNA repair disease, XP which suggested that mutations in any of 7 genes (XPA - XPG) could give rise to the disease. Two additional disease are CS (A, B, XPB, XPD, XPG) and TTD(A, XPB, XPD). Some of the genes:
◦ XPA, which encodes a protein that binds the damaged site.
◦ XPB and XPD, product displayed $5' - 3'$ helicase activity. These are found in TFIIH. Both are subunits of TFIIH which bind to the damaged strand and cooperate with each other.
◦ The XPB, product is essential for transcription initiation and patient of this show the double symptoms of XP and CS. Some mutation in XPB and XPD cause aging like: gray hair, wrinkled skin,etc.
◦ XPF, which cuts the backbone on the $5'$ side of the damage.
◦ XPG, which cuts the backbone on the $3'$ side.

10 Transcription-Coupled NER(TC NER)

The damage within the transcribed strands of genes is usually repaired more rapidly than damage in the non-transcribed strand and a different mechanism is involved in recognizing damages in transcribed strands.
• On the DNA strand NER behave as the template for transcription. like:

NER involves XPB,XPD and several other gene products. Two genes for these product are CSA and CSB. Mutation in these genes needed for DNA repair cause an inherited disorder called Cockayne's syndrome(CS).
Both CSA,CSB interact with RNA polymerase II. CSA interact with the subunit of TFIIH and CSB interact with the XPG. These interactions suggest that CSA and CSB help recruit TFIIH and XPG to sites where RNA polymerase has stalled as a consequence of encountering damage base. Defects in CSA and/or CSB lead to loss of transcription CR.
Types of DNA damage normally by NER and BER are both preferentially repaired TCR.

11 Mismatch Repair(MMR)

Mismatch repair deals with correcting mismatches of the bases, these mismatch bases are repaired by a set of enzymes to maintain Watson-Crick complementarity (AT, CG).A always bonds with T and G always with C.
Enzyme involved in both BER and NER. Recognition of a mismatch is done by several different protein including those encoded by
◦ MSH2
◦ MLH1 is required for TCR of UV-damage.

Mutations in either of these genes involved in colon cancer on chromosome 2 and chromosome 3. So these genes qualify as tumor suppressor genes.

DNA polymerase beta and epsilon used for the synthesis of the repair patches same as NER. The major problem of this system is how to recognize which of the mismatched bases is the correct one.

12 Repair Strand Breaks

Ionizing radiation and certain chemicals can produce both single-strand breaks (SSBs) and double-strand breaks(DSBs) in the DNA backbone.

- Single-strand Breaks(SSBs)

Breaks in a single strand of the DNA molecule are repaired using the same enzyme which are used in BER.

- Double-Strand Breaks(DBSs)

Two mechanism by which the cell can repair a complete breaks in a DNA molecule:
◦ One mechanism of protein that direct joining of the broken ends. This requires protein that recognize and bind to the exposed ends and bring them together for ligation. This mechanism is called NHEJ.
◦ The other mechanism that promote homologous recombination to obtain instruction from the sister or homologous chromosome for proper repair of breaks.

13 Homologous Recombination

The broken ends are repaired using the information on the intact
◦ Sister chromatid and

◦ Homologous chromosome.
The period between mitosis and synthesis of DNA is G_1, that between synthesis and mitosis is G_2.

Two of the protein used in homologous recombination are encoded by the genes BRAC-1 and BRAC-2, inherited mutation in these genes causes breast and ovarian cancers in women.

14 Cancer Chemotherapy

• Cancer is a disease of the body's cells, cells in all tissue and organs of the body constantly grow and divide to replace old and damaged cells.
• Each division requires both
◦ the replication of the cell's DNA and
◦ transcription and translation of genes require for continuous growth.
So any chemical that damage DNA has the potential to spread cancer in a body.

• Chemotherapy is the use of anti-cancer drug to destroy cancer cell.
But there has many other cell types in the cancer patient which pro-lifer rapidly
◦ intestinal endothelium
◦ bone marrow
◦ hair follicles
and anticancer drug also produce many side effects in "chemo".

References

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[5] MGMT Gene Therapy by B.Kramer, I.Alexander, P.Gunning, G.M.Cowage.