Emerging Role of Small Rnas in Double Stranded DNA Break Repair

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ABSTRACT

DNA is the primary genetic information carrier of all living cells and the maintenance of its integrity is very important for a healthy life. It is also very important for the transmission of accurate genetic information from parents to the progeny. If DNA damage or DNA alterations occur, then it leads to development of genomic instability, cancer and other genetic disorders. The DNA damage caused by internal and external factors leads to defects in the DNA metabolism. The most critical DNA lesions are double strand breaks which ultimately lead to their mutations. If the lesions are not correctly repaired, they result in the deregulation of cellular functions. So, DNA repair is one of the most important cellular functions for the removal of base pair alterations as well as single/double strand break repairs. All DNA repair mechanisms are important but the most important repair mechanism is the small Rnas that are produced at the area of double strand break which also lead to recruitment of repair proteins. For the repair of double stranded breaks, bidirectional events occur in the vicinity of the break that activate the DNA damage response by local RNA: RNA interactions.

INTRODUCTION

The activation of DNA damage check points is required for the restriction of damaged cell progression and permission for DNA repair mechanism. At the cellular and organism levels different types of DNA repair mechanism play an important role in DNA damage as they are following:-

1. Base excision repair
2. Nucleotide excision repair
3. Mismatch repair and
4.Homologous and Non-homologous end joining

The homologous (HR) and non-homologous end joining (NHEJ) both repair the double strand DNA breaks where HR uses a DNA template and NHEJ re-joins the ends without using a DNA template [7].

Another most important repair mechanism is the small RNAs mediated which are secondary recruitment of DNA damage response factor which control the DNA strand break. Double strand breaks are the most critical DNA lesions because these can turn into area of mutations and chromosomal translocations which are the well-known hallmarks of cancer. The DNA damage response (DDR) initiates a signal cascade that detect the DNA lesion and repair the DNA in cellular pathway in which, RNAs are the controller first discovered by Michelini et al., who demonstrated that the sequence specific RNA: RNA interactions organize the DDR in response to DSBs [8].

DNA Repair Mechanisms:- TYPES OF DNA REPAIR MECHANISM:-

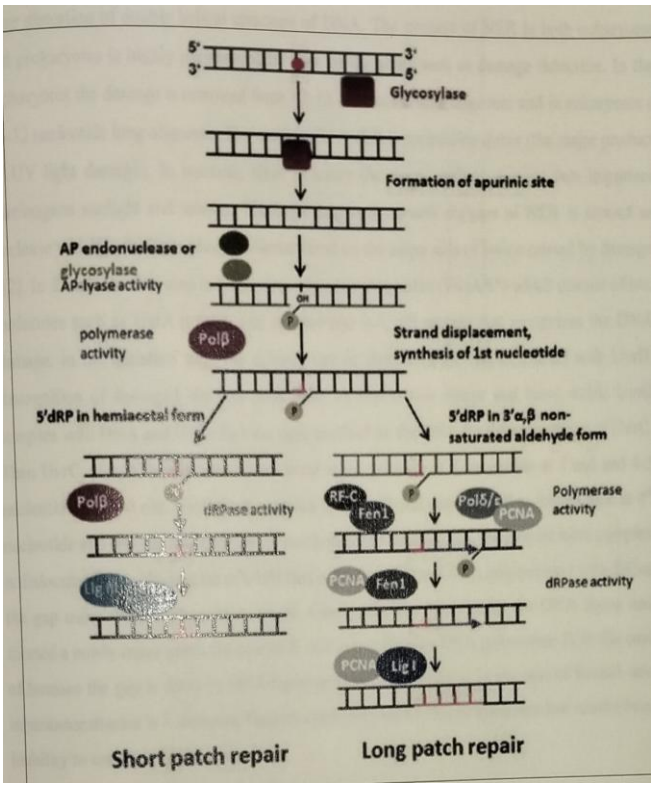
There are three types of DNA repair mechanisms which are as follows:

1.Base Excision Repair
2. Nucleotide Excision Repair

3. Mismatch Repair

Base Excision Repair in E. coli:-
Base Excision Repair (BER) in E. coli is involved in the repair of damaged nucleotide in the DNA. In this mechanism it repairs many different types of damaged bases including deaminated, abasic site (AP) site. Initial step in the BER there is the repair of single damaged base from the DNA backbone by the enzyme glycosylase, which cuts the N-glycosidic bond between sugar and base but does not break the sugar phosphate backbone of the DNA. DNA glycosylase diffuses literally along the DNA until specified kind of lesions are detected [9]. The discovery of E. coli uracil DNA glycosylase in 1974 by Tomas Lindahl marks the discovery of BER whose searched an enzyme activity that results in abasic site (AP) site bond cleavage between uracil and deoxyribose sugar. This AP site was further processed by AP endonuclease, an exonuclease, a DNA polymerase and ligase. So, DNA polymerase I binds to single strand break and synthesize the strand where, gap is created and this gap is filled by DNA ligase [10].

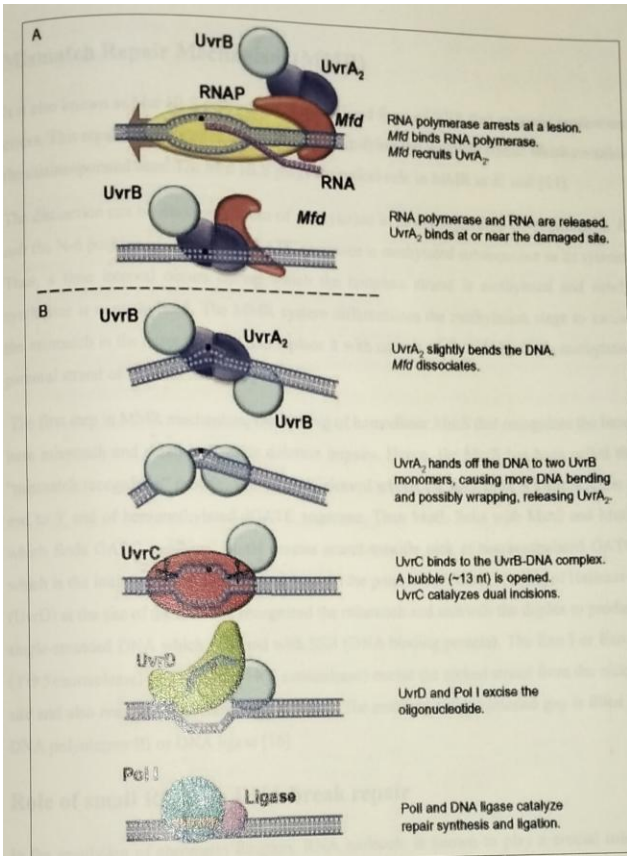
Base Excision Repair (BER) in Humans:-
BER is highly conserved from bacteria to human. It is composed of two pathways that are short patch pathway and long patch pathway that include specific enzymes. In the short patch BER, the process is initiated by DNA glycosylase which is bifunctional enzyme. The DNA glycosylase hydrolyse the phosphodiester bond to AP site at 5' with the help of apurinic/apyrimidinic endonuclease-1 (APE-1) which create a single strand break on strand with 3'OH end and another enzyme involved is DNA polymerase β which removes the deoxyribose phosphate residue (5'dRP) and place a new DNA base with DNA synthesis and gap is filled by DNA ligase III. In the long patch BER, the function of polymerase β is to fill the gap through incorporation of deoxynucleotide that is AP site by polymerase δ/polymerase ε to polydeoxynucleotide (with create no. of deoxynucleotide 5'flap) and 5'dRP residue. 5' flap is removed by flap endonuclease-1 (FEN-1) accompanied by filling the rest nick by DNA ligase [11].



[Nucleotide Excision Repair section :-
Distortion of double helical structure of DNA. The process of NER in both eukaryotes and prokaryotes the damage is removed from 12-13 nucleotide long oligomer in prokaryotes and 24-32 nucleotide long oligomer in eukaryotes. The most studied NER is pyrimidine dimer (the major product of UV light damage). In humans, NER involves the major defence against two important carcinogens sunlight and smoke. The important multisubunit enzyme of NER is termed as nuclease which hydrolyses phosphodiester bond on the either side of lesion caused by damage [12]. In E. coli, the first step in NER, depends upon a complex (UvrAB2) which consist of two molecules such as UvrA protein and another one is UvrB protein that recognizes the DNA damage. In the initiation step, the damage can be detected by UvrA in concert with UvrB. Recognition of damaged site has been done by UvrA then dissociates and leave stable UvrB complex with DNA and UvrB forms a tight scaffold on the DNA for the appearance of UvrC.

Then UvrC cleave the phosphodiester bond at the position of 8 nucleotide at 5'end and 4-5 nucleotide at 3'end site. UvrC form complex with UvrB and enable

UvrB to form a nick at 4th nucleotide at 3'end site and UvrC at 7th nucleotide at 5' end site. So, the post incision complex is dislocated by the dual action of UvrD that is (Helicase II) and DNA polymerase I with filling the gap using the complementary strand. Finally, the gap is sealed by the DNA ligase and formed a newly repair patch (in case of E. coli gap is filled by DNA polymerase I). In the case of humans the gap is filled by DNA ligase or DNA polymerase ε. In the case of human, one inheritance disease is Xeroderma Pigmentosum (XP) and Cockayne syndrome that results from inability to carry out NER system [13].



Mismatch Repair Mechanism:-

It is also known as Mut HLS pathway. It is specialized form of NER that removes replication errors. This repair system makes distinction by identifying the template strand which contains the misincorporated base. The Mut HLS plays biological role in MMR in E. coli [14].

The distinction can be made on pattern of methylation in newly replicated DNA strand. In E. coli the N-6 position of adenine in dGATC sequence is methylated subsequent to its synthesis. Thus, a time interval occurs during which the template strand is methylated and newly synthesize is unmethylated. The MMR system differentiates the methylation stage to excise the mismatch in the nascent strand and replace it with correct nucleotide by using methylated parental strand of DNA as template [15].

The first step in MMR mechanism, the binding of homodimer MutS that recognizes the base-base mismatch and small nucleotide deletion impairs. Hence, the MutS has been called the "mismatch recognition" protein. The MutS is cleaved where, the mismatch is located at the 5' end to 3' end of hemimethylated dGATC sequence. Then MutL links with MutS and MutH which finds GATC sequence. MutH creates strand-specific nick at hemimethylated GATC, which is the initiating point of mispaired base. In the presence of MutL, loading of Helicase II (UvrD) at the site of the nick and recognized the mismatch and unwinds the duplex to produce single-stranded DNA which is bound with SSB (DNA binding protein). The Exo I or Exo X (3'→5' exonuclease) or Exo VII (5'→3' exonuclease) excise the nicked strand from the nicked site and also remove the section of new strand. The resulting single-stranded gap is filled by DNA polymerase III or DNA ligase [16].

Role of small RNAs in DNA break repair:-

In the regulation of chromatin function, RNA molecule is known to play a crucial role at different levels including maintenance of genomic stability, for example, including XIST (X-inactive specific transcript) which is RNA gene. It is an early development process in mammalian female that leads to transcriptional silence of one of the pairs of two X-chromosomes. Another example is, NORAD in which the stability of genes responsible for proper segregation in cell division is controlled by NORAD. Actively transcribed DNA show.

CONCLUSION

The stability of the DNA which is the storehouse of the genetic information as well as the fidelity by which this genetic information is transmitted from parents to offsprings is very important for any cell. Any deviation in the stability or the fidelity of transmission can result in number of genetic diseases especially development of genomic instability, cancer and other genetic disorders. This dissertation work describes in detail the different types of DNA damages that occur in the prokaryotic and the eukaryotic cells as well as the different repair pathways that operate viz; Base Excision Repair, Nucleotide Excision Repair and Mismatch Repair and especially focussing on the small RNA mediated DNA break repair pathway. Small RNAs are the most novel and highly interesting candidates that are involved in the DNA repair pathways initiated by bidirectional transcription in the vicinity of the break site followed RNA:RNA interactions.

REFERENCES

1. Khanna, K. K., & Jackson, S. P. (2001). DNA double-strand breaks: signaling, repair and the cancer connection. Nature genetics, 27(3), 247.
2. Hakem, R. (2008). DNA-damage repair; the good, the bad, and the ugly. The EMBO journal, 27(4), 589-605.
3. Sinha, R. P., & Häder, D. P. (2002). UV-induced DNA damage and repair: a review. Photochemical & Photobiological Sciences, 1(4), 225-236.
4. Lindahl, T., & Nyberg, B. (1972). Rate of depurination of native deoxyribonucleic acid. Biochemistry, 11(19), 3610-3618.
5. De Bont, R., & Van Larebeke, N. (2004). Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis, 19(3), 169-185.
6. De Bont, R., & Van Larebeke, N. (2004). Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis, 19(3), 169-185.
7. Flock, O., Nielsen, O. (2004). DNA repair. Journal of Cell Science, 117, 515-517.
8. Michelini, F., Pitchiaya, S., Vitelli, V., Sharma, S., Gioia, U., Pessina, F., ... & Marti, V. (2017). Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks. Nature cell biology, 19(12), 1400.
9. Lindahl, T. (1993). Instability and decay of the primary structure of DNA. Nature, 362(6422), 709-715.
10. Lindahl, T. (1974). An N-glycosidase from Escherichia coli that releases free uracil from DNA containing deaminated cytosine residues. Proceedings of the National Academy of Sciences, 71(9), 3649-3653.
11. Fromme, J. C., & Verdine, G. L. (2004). Base excision repair. In Advances in protein chemistry (Vol. 69, pp. 1-41). Academic Press.
12. Ganesan, A., Spivak, G., & Hanawalt, P. C. (2012). Transcription-coupled DNA repair in prokaryotes. In Progress in molecular biology and translational science (Vol. 110, pp. 25-40). Academic Press.