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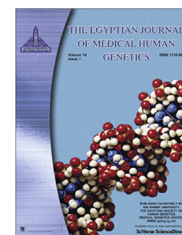

EDUCATIONAL CORNER OF THE ISSUE

Basic concepts of medical genetics, Pathogenetics, Part 4: Anti-mutation mechanisms of the human genome and human proteome

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1. Structural organization of the human genome

The peculiar structural organization of the human genome represents the first innate anti-mutation mechanism in view of the presence of large interspersed portions of **non-functional** intragenic, **introns**, and **inter-genic DNA sequences** and **segments** that can be mutated without having appreciable deleterious functional effects. In addition to **functional sequences** needed for the synthesis of protein and of regulatory small RNA species, the human genome has a considerable amount of repetitive DNA sequences, including both **noncoding repetitive DNA sequences** and **multiple copy genes** and **gene fragments**, a large number (19000–21000) of **pseudogenes**, a considerable sizable portion (about 1/6th of the total genome size) as **pyknons**, a quite large portion (nearly 40% of the total genome size) as **transposons** and large numbers of multiple copies of functional genes that share the same regulatory function and whose suppression or damage by mutation can be tolerated by other genes having the same function. These peculiar structural features of the human genome allow for occurrence of mutational events in many segments of the genome without having appreciable functional defects. Even if some of these genes or DNA sequences have important roles in genome function, their presence in multiple repetitive copies can greatly reduce, or even nullify, the consequences of mutational damage resulting from affecting many copies.

The presence of multiple copies, hundreds to thousands, of mitochondrial genes within the mitochondria of each cell is crucial in obviating devastating mutation-induced damage to these vital organelles in view of their role in production of ATP. This feature of mitochondrial genome allows for considerable burden of mutations to affect it before appreciable pathological consequences result. It is estimated that mutations affecting nearly 80% of certain mitochondrial genes might occur before pathological manifestations of mitochondrial genetic diseases make their appearance due to this multiple copy feature of mtDNA.

2. Structural features of DNA

DNA exists as a double stranded structure composed of two tightly bound strands, each strand consisting of a straight sugar-phosphate backbone with opposing nitrogenous bases linked by glycosidic linkage to the sugar of one strand and by hydrogen bonds with a complementary base on the other strand. This specific structural organization of DNA serves many purposes. It stabilizes the dynamics of the molecule, permits replication and duplication of the genetic material, protects the interiorly located bases and, most importantly, stores a template or copy of the genetic information ready for use in case of damage of the other strand. If small or gross

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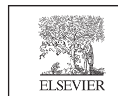

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mutational events affect important functional portions of the genetic material, repair mechanisms can restore the exact sequence of the damaged or lost or deleted parts through restoration mechanisms based on the complementary information of the other strand. Mutations leading to damage of corresponding segments of both strands represent a catastrophic event to the genome due to absence of the **sequence database** needed for the repair mechanism to define the exact base sequence of the newly synthesized segment in place of the deleted or grossly damaged segment.

3. Degeneracy of the genetic code

Degeneracy of the genetic code represents the third innate anti-mutation mechanism of the human genome. This feature permits the occurrence of **same-sense** point mutations in functional codons without changing the amino acid defined by the mutated codon. Since some amino acids, as a part of a specific protein domain, play critical roles in attaining and maintaining a correct protein structure and in mediating proper protein function, point mutations leading to replacement of these essential amino acids by other amino acids, **missense** mutations, that cannot perform the functions of the original amino acids might result in detrimental effects on the structural integrity and stability of the protein followed by deleterious consequences on physiological function of the protein. Hence, degeneracy of the genetic code allows for occurrence of many point mutations, the commonest type of mutational events and the commonest cause of genetic disorders, without changing the final structure of the synthesized protein, thus protecting against, and obviating, the pathological effects of these mutations.

4. Nuclear localization of DNA

The localization of DNA deep inside the cell nucleus represents a fourth innate anti-mutation mechanism of the human genome because it acts as a physical barrier against many mutagens that have to overcome many obstacles of cellular defense mechanisms in order to affect the nuclear genome. These defenses include the extra-cellular environment, the cell membrane, the cytoplasmic mass, the cytoplasmic enzymes and phagocytic cellular organelles and the cytoplasmic and nuclear antioxidant enzyme systems.

5. DNA-associated proteins

The DNA-associated or DNA-binding proteins, in addition to their essential roles in regulating transcriptional processes of most genes, also play fundamental roles in protecting the DNA from the damaging effects of many mutagens, in particular the free radicals that are generated during metabolic activities of the cell. They act as **physical barriers** and **biochemical buffers**, modifiers or **deactivating biomolecules** of many chemical mutagens or damaging factors that might harm the DNA. They mediate this protective role by many mechanisms including modulation of **charge transport** of oxidative agents within the DNA, limitation of DNA **helix distortion** and regulation of protein-dependent alterations in DNA base stacking [1].

6. Replication proofreading system

Preservation of genomic identity of the organism depends exclusively on an accurate replication and synthesis of two identical copies of the genome during cell division, followed by transfer, or inheritance, of each copy to each daughter cell. In this manner, all cells descendent from a parent cell have nuclear genomes identical to those of the mother cell. The majority of spontaneous point mutations of the nuclear genome is prone to occur during cell division, mostly during DNA synthesis or the replication phase of the process. The replication proofreading system acts in a prophylactic way to ensure accurate insertion or addition of the proper nucleotide to the newly synthesized strand of replicating DNA. This **prophylactic function** is fundamental to reduce the rate of inevitable replication mistakes to minimum levels that could be dealt with efficiently with the DNA repair mechanisms. In spite of the impressively fast and accurate ability of the enzymes responsible for DNA synthesis, the DNA polymerases, most of them have additional proofreading ability to ensure accurate error-free DNA replication and, hence, maintaining and preserving the stability, integrity and identity of the genome during cell division, as well as during transfer of the genetic material from parents to offspring.

7. Genetic repair systems

Genetic repair systems responsible for correcting and repairing many different types of point and small mutations that affect the genetic material, whether induced by exogenous mutagens or occurring secondary to endogenous spontaneous alterations, comprise both nuclear DNA repair system and mitochondrial DNA repair system. Genetic function and genetic repair represent two sides of one coin. Without the persevering continuous, active and effective surveillance exerted by the genetic repair systems to detect and repair the continuously and persistently occurring mutations, maintaining stability and integrity of the genome would be an impossible task. These repair systems consist of large numbers of **enzymes, proteins and related factors** that function in complementary and collaborative mechanisms along specific pathways, with each of them having a predefined role in the repair process. For instance, if mutation causes damage of a genetic segment consisting of sequence of nucleotides, an **endonuclease** enzyme cuts both sides of the damaged segment, followed by addition of proper nucleotides instead of the damaged or deleted ones by a **polymerase** enzyme, then a **ligase** enzyme joins the ends of the newly added segment of nucleotides to the original neighboring nucleotides by forming phosphor-di-ester bonds between the phosphate and the sugar of adjacent nucleotides, thus, regaining the sugar-phosphate backbone of the DNA [2].

7.1. A. Nuclear DNA repair

Nuclear DNA repair mechanisms comprise many approaches to repair mutations of DNA. These approaches include different pathways and sub-pathways according to the type, site and extent of the mutation-induced damage and also according to the stage of cell cycle affected by the mutation. They include: base excision repair (BER), nucleotide excision repair

(NER), direct reversal repair, mismatch repair, transcription-coupled repair (TCR) and recombination repair (Fig. 1).

7.1.1. Base excision repair (BER)

This repair mechanism is probably the most frequent DNA repair pathway in the cell. It is used for single strand point mutations affecting one or few bases of one DNA strand. It involves recognition of the damaged base of a nucleotide by a **glycosylase** enzyme and its removal by detaching it from the deoxyribose sugar via hydrolysis of the N-glycosyl bond. Breakage of the hydrogen bond between the damaged base and the opposing base on the complementary strand occurs, probably, via ATP-induced changes of the energy dynamics of the bond. Removal of the damaged or mutated base results in creation of abasic site or apurinic/apyrimidinic site (AP) of the DNA which are targeted by **endonuclease** and **lyase** activity to remove the damaged base(s) followed by addition of new normal base(s) by a specific **polymerase** enzyme and, finally, regaining the phosphodiester bonds and, hence, the phosphate-sugar backbone of the DNA strand by the action of DNA **ligase**.

7.1.2. Nucleotide excision repair (NER)

Nucleotide excision repair is one of the most important DNA repair systems and is highly conserved among species, though it is much more complicated in higher eukaryotes than prokaryotes. The most prominent feature of this repair system is its broad substrate specificity because it can excise DNA lesions such as UV-induced pyrimidine dimers as well as more bulky adducts of DNA.

7.1.3. Direct reversal repair

This repair mechanism can directly repair UV-induced pyrimidine dimer formation and alkylation adducts by DNA **photolyase** enzymes and **alkyltransferase** proteins, respectively. Direct reversal repair mechanisms are not followed by incision of DNA strands or resynthesis of new DNA since the changed or mutated bases are directly reverted to their original states either by light-dependent photoreactivation process, for pyrimidine dimers repair, or by use of alkyltransferase. Placental mammals do not have photolyase-dependent repair mechanisms and depend on nucleotide excision repair to correct and repair UV-induced pyrimidine dimer formation.

7.1.4. Mismatch repair (MMR)

The mismatch repair (MMR) system recognizes and corrects mismatched or unpaired bases that result from errors of DNA polymerase during DNA replication. It involves complex reactions and interactions of many enzymes, proteins and **signal discrimination factors**, probably in collaboration with the proofreading system, to recognize the mutated strand first and then to locate the site of the mismatched pair. This is followed by removal of the mutated sequence by an **endonuclease**, addition of new pair(s) by DNA **polymerase** and final regain of the DNA double stranded structure by DNA **ligases**. Post-replication mismatch repair is achieved by removal of a relatively long tract of mismatch-containing oligonucleotides, a process called long-patch MMR.

7.1.5. Recombination repair

Recombination repair mechanisms aim primarily at repairing double-strand breaks of DNA which represent the most devastating mutation-induced lesions of DNA because they can lead to loss of genetic information and chromosomal instabilities with consequent pathological alterations including chromosome breakage syndromes and carcinogenesis. Double-strand breaks can be caused either endogenously during DNA replication due to replication errors, e.g. replication fork collapse, or exogenously by, e.g. ionizing radiation. Recombination repair mechanisms consist of various steps: end resection, strand invasion, DNA repair synthesis, branch migration and Holliday junction resolution, and include, at least, two different repair pathways: homologous recombination repair (HR) and nonhomologous end-joining repair. HR repair mechanism is the accurate pathway and makes use of undamaged homologous DNA as a template for repair. Non-homologous end-joining repair mechanism directly ligates two double-strand breaks together, and although it is efficient, it is prone to loss of genetic information at the ligation sites. However, there are many anti-recombination mechanisms to suppress excessive recombination that might lead to loss of genetic information and genomic instability [1].

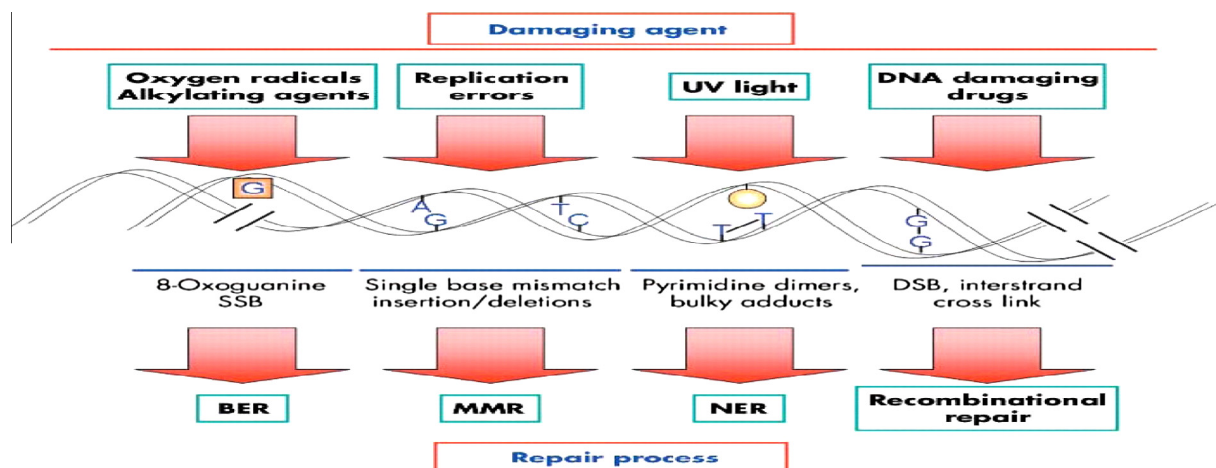

Figure 1 DNA repair mechanisms.

7.2. B. RNA repair/editing system

RNA editing refers to molecular modifications of nucleotides of RNA through chemical changes in the base makeup of the molecule. Such changes appear to be present in all three domains of life, and involve both **coding, mRNA**, and **non-coding, tRNA and mRNA,** types of small or microRNA. RNA editing occurs in the cell nucleus and the cytosol, as well as in mitochondria and is mediated by a complex repair system comprising many species of small RNA (**guide RNA**) and large protein complexes known as the **editosomes**. The pathways of RNA editing include many diverse processes: nucleoside **base modifications** such as cytidine (C) to uridine (U) and adenosine (A) to inosine (I) **deaminations**, as well as non-templated **insertions of nucleotide**. RNA editing in mRNAs effectively alters the amino acid sequence of the encoded protein so that it differs from that predicted by the genomic DNA sequence. Though mRNA editing is used in many instances to allow for synthesis by the cell of more than one protein from the same mRNA transcript, e.g. synthesis of both apolipoprotein B-100 and apolipoprotein B-48 from the same mRNA in liver cells, it can also be used to repair missense or termination mutations of the molecule which can have deleterious effects on the synthesized protein. Specific endonucleases and ligases for double stranded species of RNA have been defined in many prokaryotes and it might be just a matter of time before defining their functional counterparts in eukaryotes and human cells.

7.3. C. Mitochondrial DNA (mtDNA) repair

The pivotal role played by the mitochondrial genome in generating ATP, without which life can neither begin nor persist, in addition to many other critical metabolic and regulatory functions of mitochondrial genes, requires the presence of an efficient system for repairing mtDNA mutations. The need for mitochondrial genome repair system is further imposed on the cell in view of the high mutation rate of mitochondrial genes which lack many of the anti-mutation and protective mechanisms available to nuclear genes. Similar to the nuclear genome repair system, mitochondrial repair system includes many repair pathways: base excision repair, direct reversal repair, mismatch repair, and recombination repair. Nucleotide excision repair (NER) pathway, however, seems not to be working in the mitochondria [3].

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