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DNA Repair Mechanisms as Drug Targets in Prokaryotes

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Abstract

Nowadays, a great amount of pathogenic bacteria has been identified such as *Mycobacterium* sp. and *Helicobacter pylori* and have become a serious health problem around the world. These bacteria have developed several DNA repair mechanisms as a strategy to neutralize the effect of the exposure to endogenous and exogenous agents that will lead to two different kinds of DNA damage: single strand breaks (SSBs) and double strand breaks (DSBs). For SSBs repair, bacteria use the base excision repair (BER) and nucleotide excision repair (NER) mechanisms, which fix the damaged strand replacing the damaged base or nucleotide. DSBs repair in bacteria is performed by homologous recombination repair (HRR) and non-homologous end-joining (NHEJ). HRR uses the homologous sequence to fix the two damaged strand, while NHEJ repair does not require the use of its homologous sequence. The use of unspecific antibiotics to treat bacterial infections has caused a great deal of multiple resistant strains making less effective the current therapies with antibiotics. In this review, we emphasized the mechanisms mentioned above to identify molecular targets that can be used to develop novel and more efficient drugs in future.

Key words: DNA damage, antibiotic resistance, SSB, DSB, antimicrobial drugs, drug-resistant mutants, BER, NER, HRR, NHEJ.

1. Introduction

During the last decades, our knowledge of DNA structure and function has increased dramatically. For example, a recent publication of Wolfe-Simon *et al.* (1) shows a bacterium strain "GFAJ-1" of the *Halomonadaceae*, can use arsenic, a substance that is highly toxic to almost all life on this planet, instead of phosphorus to sustain its growth and incorporate it to its DNA. It is predicted that these bacteria may have formed more than 760,000 years ago. Until now, O_2, C, H, N, P and S have been the basic ingredients of the chemistry of life. However, the present evidence shows this bacterium uses arsenic to maintain its life machinery (1). This information has allowed us to gain a better understanding of life in general, as well as of human diseases (2,3,4). As a matter of fact, with the rise of molecular biotechnology and genetic engineering, scientists have started to develop more effective tools against diseases, targeting key components of molecular mechanisms and even targeting the origin of any living process: the genes. Nevertheless, our knowledge about DNA still remains limited, as well as the options that we need to explore on the path to developing drugs and vaccines against infectious diseases (5).

One aspect that could help us in our fight against infectious diseases is to understand DNA

repair mechanisms in pathogens and evaluate, if DNA repair mechanisms can be targets of antimicrobial drugs. So far, the DNA repair mechanisms that have been described in prokaryotes, can be classified in three main groups (6).

The first group is the direct repair, where DNA damaged part is restored to its original form *in situ*. An example of this kind of repair is photoreactivation. The DNA damage repair through photoreactivation consists of an enzymatic-dimer complex that is activated by its close-proximity exposure to near UV and visible light (6).

The second group of mechanisms share as a main characteristic that they remove the damaged section of the DNA and replace it with normal nucleotides, using the complementary strand as template to restore the sequence (7,8,9). This group of mechanism could repair damages such as mismatches, inter and intra-strand cross-links, and insertion and deletion loops originated from photoproducts of UV radiation and chemical reactions that would lead to oxidation, deamination of bases, and alkylation. It is also the first type of mechanism discovered that is totally independent of UV radiation induction. Examples of this type of mechanism include the base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR).

The third group repairs the DNA damage through recombination processes. This kind of system is called post-replication repair. These mechanisms are the homologous recombination repair (HRR) and non-homologous end-joining (NHEJ). These pathways could repair double strand breaks caused by reactive oxygen species, nuclease action or collapsed replication forks (10).

It is widely known that classical drugs and vaccines are becoming less effective due to the increase of resistant bacteria strains worldwide. The selective pressure put on these organisms and their high rates of mutation are the main causes of the increase of resistance. When a pathogen undergoes genotoxic stress, it activates specialized DNA copying enzymes that copy in an error-prone way. This process originates mutations that are in most cases lethal, but some mutations in genes linked to drug action may be beneficial. For this reason, the idea of targeting components of the DNA repair mechanisms of these pathogens is attractive. Nevertheless, we have a long way to go in our understanding of DNA repair in pathogens and the main reason for this is the complexity of DNA repair pathways. These mechanisms fight against different kinds of damage such as single strand breaks, double strand breaks, and base modifications that lead to mutations, loss of information, transitions and transversions. In addition, a huge battery of enzymes on these mechanisms is related, with very specific functions and signaling pathways that are not well-understood so far. Consequently, the inherent complexity of DNA repair mechanisms leaves us an abundance of unanswered questions, as well as the need to increase our understanding of some unknown and known processes. It is crucial that we achieve a good comprehension of key processes like damage detection, activation of checkpoints pathways, cell cycle arrest, DNA repair mechanism initiation and the mechanism pathway and signaling routes. This understanding will allow us to choose the right molecules involved in these mechanisms as drug targets against pathogens. Targeting these mechanisms could compromise the survival of pathogens to the oxidative damage caused by the immune response, and consequently decrease the proliferation of drug-resistant mutants.

The objectives of this review are: i) present the mechanisms of DNA damage repair in

prokaryotes and ii) propose potential targets within these mechanisms for the developing of novel drugs.

2. Genetic Elements that Lead to Variability and Mutations

2.1. In Prokaryotes, Plasmid DNA is Abundant: Plasmids are considered transferable genetic elements capable of replicating themselves within a host. They are double stranded and in most cases circular and are found in *Archea* and *Bacteria* domains as well as in some eukaryotes like yeasts. Their size varies from 1 to over 1000 kilobases. Each cell may harbor from one to thousands of copies of the same plasmid within it. Plasmids are a mechanism for horizontal gene transfer within a population of microbes that normally confer a selective advantage in certain environmental conditions. The process of transference of plasmids is known as conjugation. In this context, there are two main types of plasmids: conjugative plasmids and non-conjugative plasmids. Conjugative plasmids are those that contain transfer genes that perform the complex process of conjugation. Non-conjugative plasmids cannot initiate conjugation, and they can only be transferred with the assistance of conjugative plasmids. Plasmid of different types can coexist in a single cell, offering more selective pressure advantages to that cell (11). According to their function, plasmids could be classified in 5 main categories: 1) resistance plasmids, which contain genes of resistance against antibiotics or toxic substances; 2) fertility plasmids, which contain the trans-genes that allow conjugation; 3) col-plasmids, which encode for the production of bacteoricines, which are substances that kill other bacteria of the same genus; 4) virulence plasmids, which turn bacteria into pathogens; and 5) degradative plasmids, which enable the digestion of unusual substances like hydrocarbons or other contaminants (11).

2.2. Transposable Genetic Elements in Bacteria

2.2.1. Insertion Sequences (IS): Insertion sequences are small elements of approximately 1000 base pairs or less, with ends of 15 to 25 base pairs inversely repeated (IR). They have just one gene that encodes for the transposase enzyme. In many bacteria genomes exists a great amount of insertion sequences. In addition, conjugative plasmids are rich in insertion sequences. The frequency of insertion of an insertion sequence in a determined gene is about 10^{-5} to 10^{-7} (12).

2.2.2. Transposons: Transposons are sequences of 3000 to 20000 base pairs, that have at least one gene that encodes for transposase and one gene with a certain function unrelated to transposition. These other genes can encode for antibiotic resistance or heavy metals resistance (12). Two main kinds of transposons exist: The first kind is 1) composite transposons. These have two identical or almost identical insertion sequences in both ends. The transposition of these elements depends on one or both copies of the insertion sequences. The second kind is non-composite transposons, which lack the insertion sequences, and their ends are just two short sequences inversely repeated. In their central part they have the gene that encodes the transposase, and sometimes a gene for transposition regulation. They also contain genes for antibiotic resistance (12).

2.2.3. Integrons: Integrons are transposable elements with inversely repeated ends and antibiotics or heavy metals resistance genes that also have a gene that encodes for integrase. Their transposition mechanism is similar to the insertion sequences or transposons mechanisms, but in this case the integrase have allowed them to perform site specific recombination. For this reason, they have acquired genes from other

genetic elements. Integrons are usually part of greater transposons (12).

3. Molecular Basis of DNA Damage and Mutation

In nature, DNA molecules have to deal with many chemical and physical agents that are likely to cause severe damage on them and originate mutations. The two main types of DNA damage are: 1) damage that is originated within the cell because of the cell's natural physiology, known as spontaneous or endogenous damage; and 2) damage that is originated from the environment, known as environmental damage. In the spontaneous damage category, it could be mentioned the mismatches that arise during DNA replication, the damage resulting from spontaneous modifications of DNA (incorporation of uracil, deamination of bases, depurination and depyrimidination), and oxidative damage caused by oxygen reactive species (ROS). In the environmental damage category, they are the base damage and strand breaks caused by ionizing radiation, photoproducts originated by UV radiation and the alkylation of bases caused by alkylating agents. In addition, genetic transposable elements could cause mutations in bacterial genomes (13).

In the following sections the molecular mechanisms of these different kinds of damage were discussed.

3.1. Mismatches: Mismatches are mainly originated when DNA polymerase commits an error that is not corrected by the 3' exonuclease activity. The incorrect pairing occurs because of rare and less stable forms of nitrogenous bases appearing during replication: the tautomers (mentioned above). The proton's change of position alters the bonding properties of the base. Table-1 shows the bonding properties of tautomeric forms of nitrogenous bases.

Table 1. Bonding properties of tautomeric forms of nitrogenous bases.

Tautomeric Form	Behaves as	Bond Formed
A (Imine)	G	A-C
G (Enol)	A	C-A
C (Imine)	T	T-G
T (Enol)	C	G-T

Transitions can also be caused by deamination of cytosines. Deamination turns cytosine into uracil, which can then base-pair with adenine. Therefore, in the next round of replication, the complementary strand containing the adenine will serve as a template to pair a thymine, originating a transition G:C to A:T. The Deamination can occur spontaneously, but it appears less frequently in double stranded DNA than in single stranded DNA. Normally, uracil is not added to DNA due to the process of dUTPase being encoded by the *dut* gene, but strains lacking this gene have higher chances to incorporate uracils originated from the deamination of cytosines (13).

3.2. Depurination and Depyrimidination: Depurination and depyrimidination are alterations of the DNA structure, in which a purine or a pyrimidine is removed respectively by hydrolysis of the glycosidic bond from the deoxyribose sugar. After this phenomenon, the absence of information from the complementary strand will lead to mutation because the BER (base excision repair) will add an incorrect base. This would originate transition or transversions mutations (change of a purine-pyrimidine pair for a pyrimidine-purine pair). The main cause of depurination and depyrimidination is the presence of endogenous metabolites going through chemical reactions. Depyrimidination occurs less frequently than depurination. This happens because purine is a susceptible group, and the anomeric carbon is especially reactive towards nucleophilic substitution, making the

carbon-purine bond longer and weaker, and therefore susceptible to hydrolysis (13).

3.3. Oxidative Damage: Oxidative damaged is caused when reactive oxygen species (ROS) attack the DNA (14). ROS are generated in cells as byproducts of respiration and by ionizing radiation. The two main radicals involved in DNA damage are the peroxide radicals (H_2O_2) and the hydroxyl radicals (OH^-). A large variety of chemical derivates of the nitrogenous bases are produced when DNA is attacked by ROS. For example, OH^- radicals attack C5=C6 double bonds. If this occurs in thymine, it leads to formation of thymine glycol that could block DNA replication (15). Another common example of ROS's effect is the conversion of guanine into 7, 8-dihydro-8-oxoguanine which is mutagenic because it mispairs with adenine. Figure 1 shows examples of DNA damage induced by reactive oxygen species.

3.4. Environmental Damage: Several environmental factors could damage DNA. The first one is ionizing radiation. Eighty percent of ionizing radiation in cells takes electrons from water forming the H_2O^+ radical. If oxygen is present, hydroxyl and peroxide radicals are also formed. Radicals formed by ionizing radiation cause the same base damage that is caused by radicals formed from the metabolism of the cell. Furthermore, ionizing radiation can cause damage to the sugar residues. Such damage leads to single or double strand breaks, which can result in cell death or mutagenesis if they are not repaired. A break in one strand is repaired easily using the opposite strand as a template. Breaks in both strands are repaired as single breaks if they are well spaced. But if breaks in both strands are directly opposite or separated by few base pairs, it leads to a double strand break that separates chromatin (16).

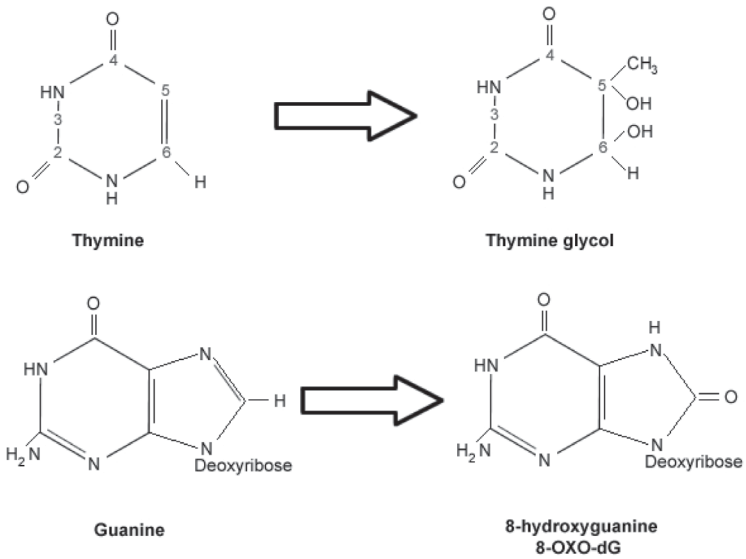

Fig. 1. Examples of DNA damaged bases induced by reactive oxygen species. Upper panel shows the conversion of thymine into thymine glycol, this form could block DNA replication. Lower panel shows the conversion of guanine into 8-hydroxyguanine which is mutagenic because it mispairs with adenine.

The second main environmental factor that damages DNA is UV radiation. Photoproducts are most efficiently induced with 254nm UV light. UV radiation produces cyclobutane pyrimidine dimers. These dimmers are form when adjacent pyrimidines are covalently linked through carbons 5 and 6. This damage interferes with DNA transcription. The most common pyrimidine dimer is the thymine dimer (17).

The last environmental factor that leads to DNA damage is alkylation. An alkylating agent is an electrophilic compound with high affinity for nucleophilic centers in organic macromolecules. We can distinguish between mono-functional alkylating agents, which have one reactive group and can react with one nucleophilic center in DNA, and bi-functional alkylating agents, which have two reactive groups and can react with two sites in DNA. Alkylation of DNA can result in mutations in several ways. The addition of alkyls groups will distort the DNA double helix. As well, alkylation can lead to mismatches that result in transitions.

3.5. Mutations Caused by Genetic Transposable Elements: The insertion of a transposon or an insertion sequence originates the inactivation of the gene because of the reading frame shift. After the insertion, deletions of the transposable element and adjacent genetic sequences can occur. On the other hand, two insertion sequences of the same type, located at a certain distance can go through recombination of the inversely repeated ends or translocation of the genetic material between them (12).

4. Single strand break DNA repair mechanisms

Single strand breaks is the most common type of DNA damage that is found in cells. SSB have an arising frequency in tens of thousands per cell per day. SSBs consist in one or more discontinuities in a single strand of the DNA double helix. The most important consequence of SSB in chromosome is the collapse of the DNA replication fork in the S phase during the cell cycle leading in some cases to the arise of double strand breaks (18).

4.1. Base Excision Repair in prokaryotes: Base excision repair is a cellular mechanism that fixes damaged DNA throughout the entire progression of the cell cycle. This mechanism exists because there is a high spontaneous-mutation rate present in organisms during DNA replication. There also exist mutagens in the environment which can further increase this inherent mutation rate that all organisms posses. BER is important for removing damaged bases that could cause mutations by incorrect pairing or lead to breaks in DNA during replication. It is more specifically directed to repairing single-strand breaks of DNA. This means that the damaged base is located in only one of the helixes; it is eliminated and then correctly synthesized using the complementary base on the other helix. The other mechanisms that specialize in single strand repair are the nucleotide excision repair system and mismatch repair system. These damages affect the fidelity of DNA replication. Depending on the nucleotide to be removed, the pathway has different approaches and variations but the specificity is conferred by the DNA-N-Glycosylase to be utilized (19).

4.1.1. BER mechanism in E. coli: There are several enzymes that are documented for *E. coli* to continue with its repair mechanism and correct DNA replication. In *E. coli*, 2, 6-dihydroxy-5N-formamidopyrimidine-DNA glycosylase (also known as Fapy or Fpg DNA glycosylase) removes the mutagenic adduct from DNA. Fpg is a DNA glycosylase that removes Fapy and 8-oxo-G from DNA (20,21,22). In *E. coli*, several DNA repair enzymes, known as the GO system,

prevent mutagenesis caused by 8-oxo-G. This system consists of: MutM [2, 6-dihydroxy-5N-formamidopyrimidine (Fapy)-DNA glycosylase, Fpg], MutT (8-oxo-dGTPase), and MutY (adenine-DNA glycosylase) (23,24). Predominantly, ROS has proven to cause DNA damage and affect damage repair. *E. coli* has two families of AP endonucleases: the family of endonuclease III and the family of endonuclease IV. Endonuclease III is coded by the *xthA* gene and endonuclease IV is coded by the *nfo* gene (25,26,27). These two endonucleases have great relationship with the Fpg protein which removes damaged purines from DNA (28). If either of these proteins is eliminated or bypassed, it could potentially hinder the organisms' ability to manage the damages in the DNA. For this reason, these proteins are excellent candidates for continued study.

Several mutagens affect DNA repair efficiency. For example, hydrogen peroxide (H_2O_2) causes sensitivity in the gene (*zthA*) that code for endonuclease III (29). This generates many AP sites that, if not eliminated by the DNA-glycosylases, will result in the decrease in the survival rate of *E coli* (30). Endonuclease III (which makes up for 90% of nucleolytic activity) has the function of AP-lyase and it cleaves 3´ to the AP site leaving a 5´ phosphate and a 3´ ring opened sugar. H_2O_2 generates free radicals that cause DNA strands to break, leaving 3' phosphate groups. Once these products are formed, they block the action of polymerases and are susceptible only to endonuclease III (Fig 2). *E. coli* mutant strains for the *zthA* gene (31), have demonstrated that, with these conditions, the survival rate for cultures subjected to treatments with H_2O_2 decreased. Comparatively, *nfo* mutants have suggested that endonuclease IV also bears some responsibility in the repair of lesions caused by this agent, but the bacteria depends more on the function performed by endonuclease III (32). It has been described recently that endonuclease IV plays a role in an alternative pathway to classic BER, called nucleotide incision repair (NIR), in which it cleaves DNA generating terminus which constitutes the DNA polymerase target. Therefore, the advantage of this pathway is avoiding the genotoxic intermediates generated in BER mechanism (32).

4.2. Nucleotide Excision Repair in prokaryotes: The first evidence of the existence of the NER repair system is shown in the 1960s, when researchers observed the excision and damage repair induced by UV in bacteria. This damage is mainly cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts (6-4PPs). If these damages persists in the bacteria genome, eventually can cause the impediment of DNA replication and transcription that can cause cell death. For this reason, cells posses several mechanisms that contribute to survival after UV irradiation. These mechanisms include UV-absorbing pigmentation, repair or removal of the UV photoproducts, cell-cycle checkpoints and some grade of damage tolerance that permit the replication of the cells even when damage is still unrepaired (33).

In 1965, Howard-Flanders *et al* (34) showed that on *E. coli* mutant strains and they found evidence suggesting that, the repair of thymine dimers and other damages caused by UV radiation in the DNA helix is controlled by three genetic loci: *uvrA, uvrB, uvrC* (34). In the first instance, the expression of *uvrA* and *uvrB* were only related with the SOS response to damage caused by mutagenic agents to DNA. However, more recent observations determined that the inducibility of UvrC expression is also regulated by the SOS response at the cellular level (35). All three *uvrA, uvrB* and *uvrC* are regulated in the same way and they are jointly involved in the synthesis of the UvrABC

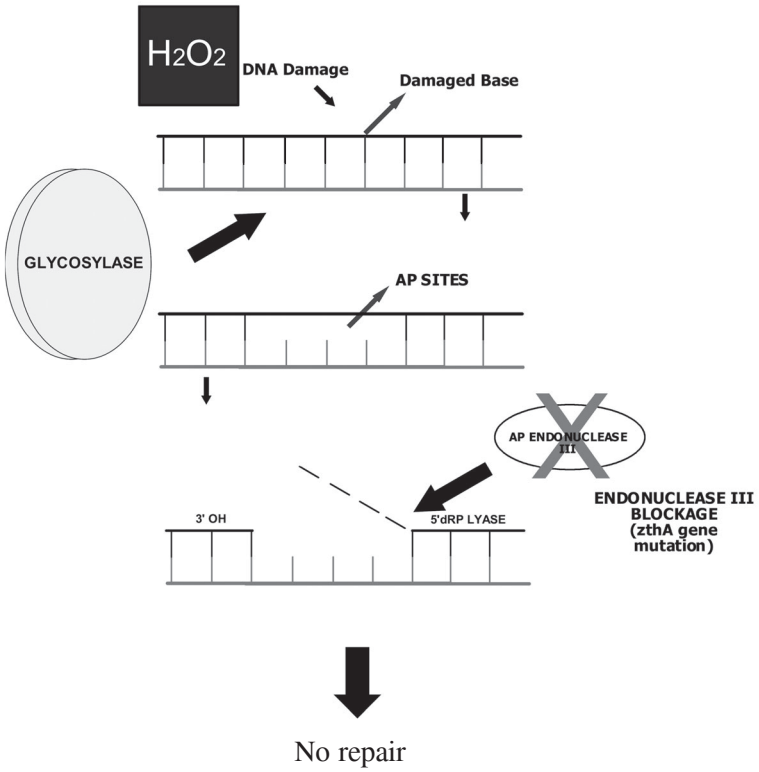

Fig. 2. Proposed target to inhibit the base excision repair mechanism. DNA is damaged by several agents, among them, hydrogen peroxide. This produces damaged bases that are excised by a DNA glycosylase which generate an apurinic/apyrimidinic site (AP site). The only way that *E. coli* can repair a damaged caused by H_2O_2 is through endonuclease III and endonuclease IV. If the synthesis of either of these enzymes is inhibited, the bacteria is incapable of remedying the damage caused by this agent.

endonuclease (35,36). The UvrABC proteins recognize and cleave damaged DNA in a multistep adenosine triphosphate (ATP)-dependent reaction. Bacteria undergoing repair have such a late requirement for the *uvrC* gene product, unless UvrC plays a semi detached, independent role in the detection and processing of DNA damage (37). It has been showed that the UvrABC catalyzed *in vitro* repair reaction can be separated into two consecutive steps: an ATP-dependent UvrAB-catalyzed binding to the damaged template, followed by an ATP-independent UvrC-catalyzed endonucleolytic step (37). Sharma and Moses (37) concluded from repair experiments in permeable cells, that the UvrC protein might be required late in the incision step. *In vitro* studies on DNA incised by *Micrococcus luteus* pyrimidine dimer-N-glycosylase, revealed that extracts from UvrC + cells catalyzed repair replication by DNA polymerase I (38).

The studies mention above has shown that the expression of *uvrA* and *uvrB* genes responsible for the NER is regulated by the SOS system, which acts in the presence of agents that cause extensive damage to double-stranded DNA (34,35). In the first instance, the expression of

uvrA and *uvrB* were only related with the SOS response to damage caused by mutagenic agents to DNA. However, more recent observations determined that the inducibility of *uvrC* expression is also regulated by the SOS response at the cellular level (35,37).

4.2.1. NER repair pathway

4.2.1.1. NER repair in prokaryotes: The a function in DNA replication. The UvrABC complex recognizes DNA damage and repairs it in a series of ATP-dependent reactions. Roughly, the NER system consists of three main steps: recognition of damaged DNA, excision and repair, and finally ligation of the repaired segment (40). Although NER is conserved in prokaryotes, archae bacteria and eukaryotes, the simplicity of the proteins make a difference in this system in relation to humans and prokaryotes. In prokaryotes, the NER acts in a much more simple way. It only requires the presence of three enzymes: UvrA, UvrB and UvrC, which make up the UvrABC complex. (40)

The first step is the recognition of the DNA lesion. It is the most important, as it has proven to be the key step for success of the entire repair system. Unlike other repair systems, NER is the only one in which recognition is given to the open threads. Initially the UvrA dimer recognizes sites of DNA damage and causes a twist in the double strand of DNA that allows the binding of UvrB to form a complex called UvrA2B. Given the characteristics previously studied in the recognition of lesions in DNA by this complex, it is thought that recognition is not only chemically, but transcends to recognize the impact that these injuries have in the flexibility of DNA (changes in DNA topology) (Fig. 3).

One of the features that make the NER one of the most important repair systems and one of the most used of all is because of its great ability to recognize many different types of lesions in double-stranded DNA. It has a wide range of injury recognition. The mechanism used is uncertain but there are many speculations. It has been supposed that the UvrA does not recognize the injury itself, but rather the distortion of the double helix of DNA induced by the lesion's presence. This helix distortion may include the disruption of base pairing as well as the bending of DNA strands (41). UvrA protein has two DNA binding sites, one located at the N-terminal and the second located at the C-terminal. It has been shown that this protein binds to damaged DNA both in the presence or absence of nucleotide cofactors, thus forming a double mark on the string that marks the site of injury that needs to be repaired. The size of the footprint is about 33 bp (42). For a proper recognition process, the functionality of the ATPase domains of the system is vital, so the UvrA protein can successfully recognize the lesion in the DNA.

After the lesion sites are detected, both enzymes work together in an ATP-dependent reaction, resulting in a stable complex between UvrB and damaged DNA. The UvrA dimer dissociates the complex and returns to be used for further recognition of DNA lesions (39). In this way the complex is formed between the injured DNA pre-incision and UvrB protein.

After this occurs, the UvrC endonuclease recognizes the pre-incision complex formed by the damaged DNA and the UvrB protein, and together they cut the piece of DNA chain damaged. The process is this; make two incisions, one in the eighth phosphodiester bond in the direction 5' downstream to the injury, and the second incision at the fourth phosphodiester bond 3' upstream the injury. The first incision is made in the last 3' through the UvrB while the second takes place at the 5´ and goes through the UvrC. The resulting fragment is finally removed. (43) While UvrC protein dissociates

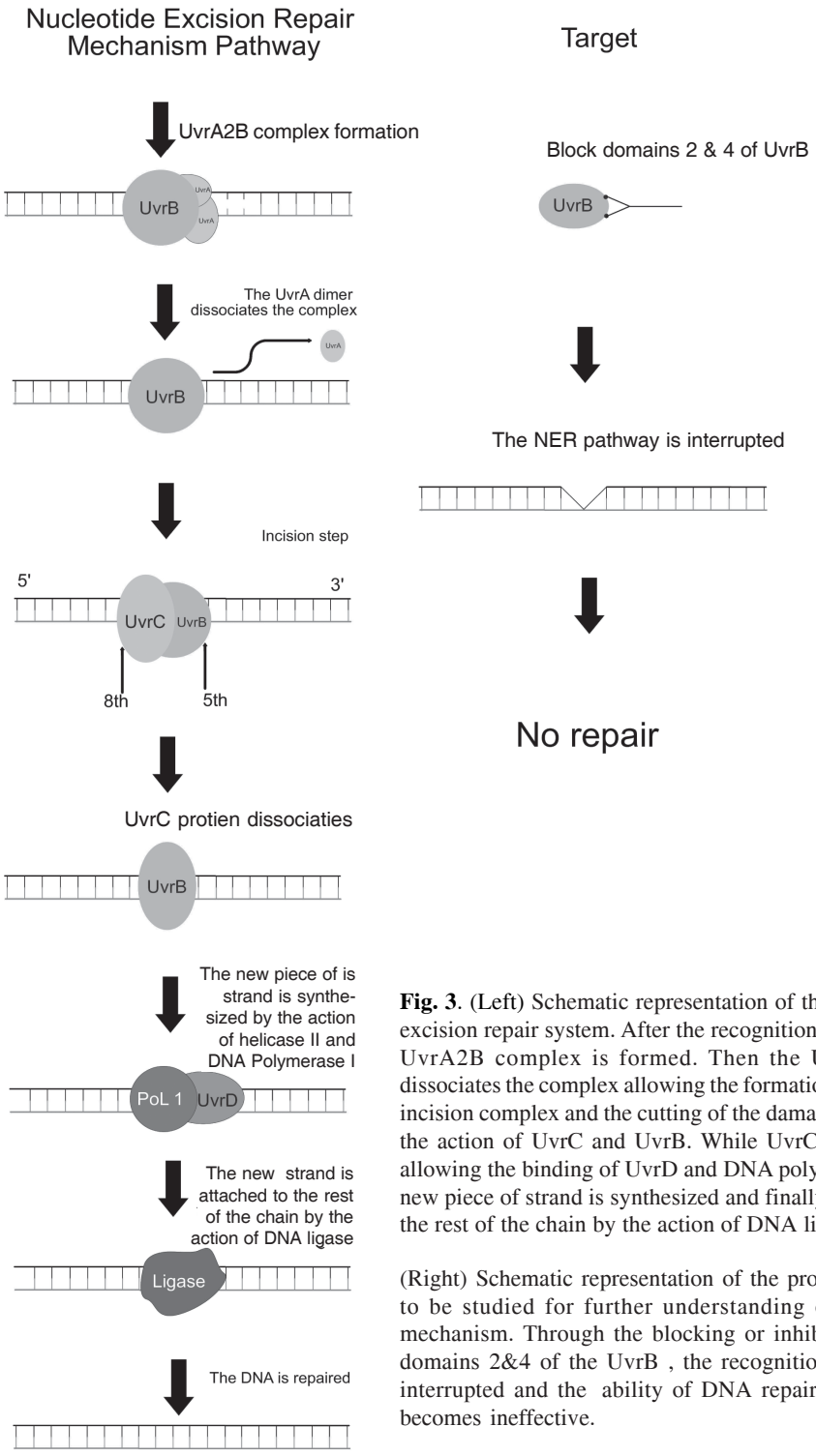

Fig. 3. (Left) Schematic representation of the nucleotide excision repair system. After the recognition process, the UvrA2B complex is formed. Then the UvrA dimer dissociates the complex allowing the formation of the pre-incision complex and the cutting of the damaged DNA by the action of UvrC and UvrB. While UvrC dissociates allowing the binding of UvrD and DNA polymerase. The new piece of strand is synthesized and finally attached to the rest of the chain by the action of DNA ligase.

(Right) Schematic representation of the proposed target to be studied for further understanding of the NER mechanism. Through the blocking or inhibition of the domains 2&4 of the UvrB , the recognition process is interrupted and the ability of DNA repair using NER becomes ineffective.

allowing the binding of UvrD (a helicase II), the UvrB is displaced by DNA polymerase. The new piece of strand is synthesized by the action of UvrD and DNA polymerase I restructuring the new strand in the presence of dNTPs. The new strand is attached to the rest of the chain by the action of DNA ligase.

***4.2.2. NER** in Mycobacterium* sp.: Among some of the pathogens that use this pathway to repair their DNA is the *Mycobacterium* genus. The studies previously done in *Mycobacterium tuberculosis* and *M. smegmantis* lead to the conclusion that the NER repair system is very important in the *Mycobacterium* genus to repair their DNA resulting from external damage (44).

4.2.2.1. Mycobacterium* sp. *Case: *Mycobacteria* are an important group of pathogens that affect humans. It resides in host macrophages, and due to the hostile conditions in which lives it can be considered one of the most successful pathogens. It is prone to survive in a hard environment of macrophages, supports low temperatures, low pH, and environmental stress caused by external factors like UV exposure, among others (44). Additionally, in recent years the coinfection rate has grown between *M. tuberculosis* and HIV, as well as the emergence of strains resistant to the drugs being used (45).

It is well known that DNA is a biological target for ROS and NIS, in addition to the many toxic radicals that are mutagenic. The DNA of the pathogen within the host is exposed to all kind of damages and injuries, which jeopardizes the integrity of its genome. It can be seen clearly how the DNA repair systems are critical for virulence and survival of intracellular pathogens so they can maintain the integrity of its genome and to remain within host cells (45). Previous studies have established the role of DNA repair systems in the success that has this pathogen to persist in host macrophages (45). By means of gene knock-out it has been determined that the strains that are deficient in NER are the most sensitive to situations that cause DNA damage. For this reason it is suggested that the NER repair system is extremely important in the genus *Mycobacterium* (46). Through studies conducted in *Mycobacteria* has indicated that UvrB is the central part of the repair system. In studies conducted by Darwin *et al* (47) demonstrate that UvrB-deficient strains of *M. tuberculosis* showed a marked non-survival pattern in mice (47).

4.2.3. UvrB as drug target: Studies by Deisenhofer *et al* (48) are the first to elucidate the structure of one of the components of the NER. They elucidated the structure of the enzyme UvrB from *Thermus thermophilus*. The enzyme UvrB consists of three domains: H1, H2, and P1. The H1 and H2 domains are very similar and they share the same aminoacid sequence. Both are connected by a linker or connector. They have a large central beta sheet flanked by many alpha helices (48).

Helicase activity has been held by the UvrB because the H1 and H2 UvrB domains are similar to those that have helicase activity. Therefore it should have the ability to "sense and scan" double-stranded DNA for lesions. But we know the limited ability of the ATPase activity in UvrB, merely moving about 22 base pairs depending on the DNA melting temperature. For this reason some authors describe this activity not as a helicase but as "destabilizing thread" (48).

The UvrB protein repair system plays an important role in the process of recognition of the injury (49). Their interaction with UvrA and DNA strands causes a series of conformational changes in the structure of the double chain waste leaving the arms uncovered in A1 and A2 of the UvrB. This process is decisive in the recruitment of UvrC to the DNA injured (50). We can notice how UvrB actively participates in the three main

steps that make up the NER, and thus can be regarded as a central molecule in this repair system. For this reason we can consider the UvrB to be a good target to inhibit the NER repair system and thus be used as a DNA based antimicrobial target. As previously mentioned in the text, the enzyme UvrB is actively involved throughout the process of repair system for excision of nucleotides. It interacts with all other key parts in the system. Ranging from the recognition phase, pre-incision complex, cleavage and sealing piece has been cut by the DNA polymerase and ligase.

Due to its vital importance in the system, reversing the activity of the NER should be viewed as a realistic and possible objective (Fig. 3). It can be used as a therapy against pathogens that use this pathway to maintain the integrity of its genome inside the host. Because of the importance of UvrB and the role it plays in NER, using it to inhibit the repair system in pathogenic prokaryotes might be a good objective that should be considered.

5. Double strand breaks DNA repair mechanisms

The DBS can cause cell death and cellular transformation, and deleterious mutations in bacteria (51,52). In addition, antibiotics can generate DSB, hence activating the DNA repair mechanisms in bacteria, a situation that is not desirable in pathogens. The treatment of bacterial infections diseases has become a difficult task since the arising of bacteria antibiotic resistance. In the case of pathogenic bacteria, not only the antibiotics apply selective pressure, but also the immune response plays an important role (53).

The two main mechanisms that repair the DSB are the HRR and the NHEJ. The HRR is considered a more accurate repair mechanism, because the homologous sequence is used to direct a faithful repair of the DSB. The HRR is triggered during the sister chromatids phase of the cell division. In contrast, the NHEJ repair acts in the G1 phase and does not require a DNA template for the DNA repair (54). These two pathways, HRR and NHEJ are described as "error-free" and "error-prone" respectively (55).

5.1. Homologous Recombination Repair in prokaryotes: The comparative and evolutionary studies of bacterial homologous recombination systems, realized by Rocha *et al*, have shown that almost all bacteria groups possessed homologous recombination machinery (56). Studies insight that the homologous recombination repair mechanism is highly conserved among prokaryotes, archae and eukaryotes. In the repair mechanism, a variety of enzymes work together to perform the DNA reparation. Of these enzymes, the most conserved is the RecA, which is a recombinase. The other enzymes that participate in the DNA repair are the RecBCD holoenzyme, and the enzymes that resolve the Holliday junction. The main functions of RecA are: strand exchange, and promoting the annealing of the 3´ single strand DNA from the broken chromosome with its homologous (57,58).

5.1.1. Homologous Recombination repair mechanism: In the mid 1940´s, the homologous recombination mechanism is first described in *E. coli*. And so far three main pathways have been described: the RecBCD, RecFOR and the AddAB. The RecBCD (primary pathway), which promotes the repair of DSB, the RecFOR (secondary pathway), which is involved in the ssDNA gaps repair and which is supposed to work when the primary pathway is inactive, and last but not least the AddAB pathway. Since the re-combinational repair mechanism is highly conserved among bacteria, the two pathways are present in very different species like *E. coli* and *Bacillus subtilis* (56,58,59). Both pathways

provide the ssDNA with RecA and permit the invasion of the homologous molecule. Although these pathways have the same function, they repair different types of DNA damage (56).

5.1.1.1. RecA, the HRR central protein: RecA is an important protein that participates in the DNA SOS induction mechanism, DNA repair and DNA recombination. Unlike the RecBCD complex, the RecA enzyme is not part of an operon (59). The RecA protein (and RecA homologs) is highly conserved in prokaryotes and in other organisms, and this protein catalyzed the key step of strand invasion and strand exchange in HRR (56). An important step in the RecA function in the cell is the ATP hydrolysis in a DNA dependant mode (60). To induce the conformational rearrangements for the strand exchange reaction, the RecA helps pairs the ssDNA with its homologous duplex DNA and hydrolyzes ATP, and this reaction produces two new DNA molecules (53). The RecA is a 38kDa enzyme and functions as part of a helical nucleoprotein filament; in addition, the main function of this protein is the search of homologous sequence both catalytically and stoichiometrically (59). This repressor binds to a promoter that activates the transcription of nearly 40 genes that are involved in repairing DNA damage (53,61). In *E. coli,* RecA expression is up-regulated by events that challenge the integrity of the bacteria genome. When the HRR is activated, this pathway can follow two different ways: de-repression of the SOS regulon or re-combinational DNA strand exchange (53). If errors are generated during the DNA replication, RecA will inhibit cell division by SOS induction. Hence, damaged DNA activates RecA and its activated form catalyzes self-cleavage of LexA. The SOS response increases the ability of the cell to repair DNA damage and delays cell division (59).

5.1.1.2. RecBCD pathway: The central step in this pathway is the synapsis between the homologous DNA molecules. The RecA enzyme catalyzes the DNA strand exchange, and forms a filament on the ssDNA, which is the active species in the exploration of homology. Besides, it is an important component in future invasions of the homologous duplex DNA. Therefore, a DNA lesion that requires recombination repair must first be processed in ssDNA by the action of helicase and nuclease. This complex has many biochemical activities such as DNA binding, DNA helicase, RecA binding, DNA dependent ATPase, ssDNA endonuclease, Chi regulated nuclease, dsDNA exonuclease, and helicase activity (62).

RecBCD is a heterodimer and consists of three different polypeptides. The RecB is a 134kDa protein, which is a DNA dependent ATPase, a weak helicase that operates in the 3'-5' direction. In addition, RecB plays an essential role in the RecA loading mechanism onto Chi containing ssDNA. The Chi sequence (5'-GCTGGTGG-3') is a regulatory sequence and a critical cis-acting DNA element. RecC can stimulate the ATPase and helicase activities of the RecB protein. Further, RecD has two important activities, which are the ssDNA dependent ATPase activity and the 3'-5' DNA helicase activity (51,62). The RecBCD substrate is a free blunt or almost blunt duplex DNA end. The RecBCD enzyme possesses a helicase and nuclease function. This enzyme complex initiates the DSB repair by converting a blunt dsDNA end into a duplex molecule leaving a 3‘ terminated ssDNA tail. RecBCD directs the RecA protein onto this ssDNA (56,62).

The RecBCD holoenzyme binds to the damaged dsDNA. This enzyme unwinds and degrades DNA from one end until it finds the Chi sequence site in the correct orientation (63). When the RecBCD enzyme recognizes the Chi

site, its function is modified, stops degrading the 3'ending strand and begins to produce a 3'single strand extension. These extensions are needed for the RecA ssDNA strand invasion, and this step is the central event in HRR (64). Then the RecA protein binds to the Chi terminated 3' ssDNA tail (56,63). When this loading mechanism is not present, the resulting ssDNA product is rapidly and tightly bound by ssDNA binding protein, which binds ssDNA nonspecifically. When the RecA nucleoprotein filament is formed, the next step is the most crucial phase of HRR, which is the search of the homologous sequence donor, where the RecA enzyme performs a scanning process of the whole genome looking for the sequence homology (64). This eventually results in loading of replication DNA helicase (62). The strand exchange reaction is propagated uniquely 3'- 5' relative to the ssDNA substrates (Fig. 4) (65).

Another protein that participates in the HRR, is the single strand DNA binding protein (SSB). This protein can inhibit or enhance the RecA filament formation (65). The filament assembly on ssDNA occurs with distinct nucleation and extension steps with extension proceeding 5'- 3'. During the DNA strand exchange the SSB binds to the displaced strand of the duplex substrate. The effect of SSB depends on when it's added (65).

So far, two independent enzymatic systems for DNA junction in *E. coli*, have been described: the RuvABC resolvasome and the RecG helicase. In addition, it is found that the DNA interaction mechanisms of these systems in some measure complement each other, even though they are quite different, since mutants with single mutations in one or the other system show only a fair defect in recombination repair. This suggests that there exists more than one way of DNA junction resolution *in vivo* (59).

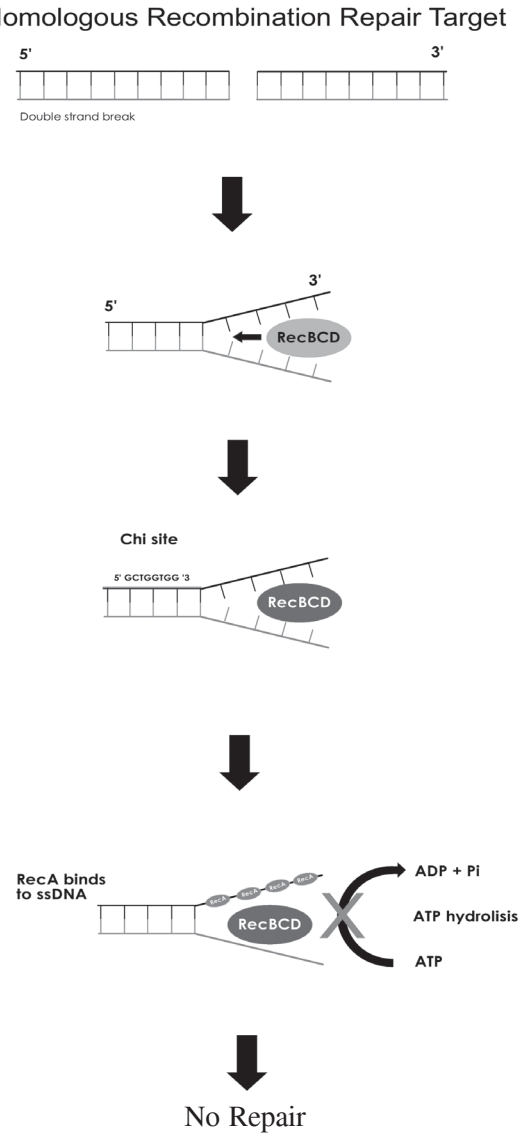

Fig. 4. The RecBCD holoenzyme binds to the damaged dsDNA. Then this enzyme began to unwind and separates both strands until it finds the Chi site in the correct orientation, converting a blunt dsDNA end into a 3, terminated ssDNA tail. When the RecBCD enzyme recognizes the Chi site, its function is modified, stops degrading the 3, ending strand and begins to produce a 3, single strand extension. Then the RecA protein binds to the Chi terminated 3, ssDNA tail. RecA is activated in the presence of ssDNA and ATP. A RecA ATPase inhibitor could be a adjuvant for the inhibition of the homologous recombination pathway.

The biochemical activities of RuvABC proteins and the ways they interact with DNA junctions, both structurally and functionally are now well characterized. In the HRR, the RuvC and RuvAB proteins catalyze the resolution of the Holliday junction and branch migration (56).

5.1.1.3. RecFOR pathway: It has been found that in the RecFOR pathway, the RecF, RecO and RecR, interact with each other, but do not form an enzyme complex as the RecBCD holoenzyme. The RecJ enzyme also interacts in this pathway, and it is important in the RecBCD pathway. This enzyme is an exonuclease that helps extend the ssDNA region when is needed. It degrades the 5' strand from a duplex DNA end during unwinding by the helicase RecQ, generating a 3' overhang. In *E. coli*, RecJ is an exonuclease specific for 5'ssDNA. RecFOR, helps RecA bind to the ssDNA (66,56). In addition, as in the RecBCD pathway the RecA protein catalyzes the strand exchange (56). In the RecF pathway, RecQ and RecJ, work in conjunction for the DNA repair (62).

The RecO protein has diverse functions, like stimulation onto the RecA's conjunction of filaments, and onto SsbA coated single stranded DNA. This protein also modulates RecA mediated DNA strand exchange, and promotes the annealing of complementary DNA strands. In addition, RecO possesses an important role in the RecA plasmid transformation (67).

The single strand binding protein, SsbA is essential for cell proliferation, because it can inhibit the spontaneous annealing of complementary DNA strands (67). SsbA facilitates the RecO mediated strand annealing by means of the accumulation of non productive ternary complexes. How RecO mediates the DNA strand annealing occurs, could be as follows: The ssDNA-SsbA-RecO ternary complex, it is formed when the SsbA binds to the ssDNA and recruits the RecO. After the formation of the ternary complex, RecO interacts with SsbA-ssDNA and with itself leading to the formation of bridge structures, and the RecO protein decreases the half life of the SsbA-ssDNA complex. When RecO binds to the naked ssDNA, it distorts the ssDNA structure and prevents the SsbA binding or relieves it from ssDNA (67).

Studies performed by Manfredi *et al* (67), suggest that RecO has three main activities coordinated by SsbA: 1) can recruit RecA onto SsbA coated ssDNA; 2) can modulate the extent of RecA mediated DNA strand exchange; and 3) bridges SsbA coated ssDNA molecules, when the complementary promotes annealing (67). The strand annealing mediated by RecO is critical for the RecA filament extension and strand exchange during recombination mediated by RecA (67).

5.1.1.4. AddAB pathway: The AddAB pathway which is found in *Bacillus subtilis*. The AddAB is a nuclease/helicase protein complex that generates a ssDNA region at the DSB, and acts upstream the RecA. Downstream RecA, the RecG and RuvABC complex are involved in the formation of the Holliday junction and the crossover resolution (57). The AddA subunit of the AddAB protein complex presents homology regions with the RecB, which contains an N-terminal helicase domain and a C-terminal nuclease domain. The AddB subunit also contains a conserved nuclease domain at its C-termini. The helicase and nuclease domains of the AddAB, coordinate the bindings of dsDNA ends, and before the Chi sequences are encountered by the RecBCD, the AddAB catalyzes the unwinding and degradation of both DNA strands. The 3' strand cleavage activity ends when the AddAB recognizes the Chi sequence and the degradation of the opposite strand in the 5'- 3'. direction is not affected (58). Studies realized so far have elucidated different

HRR pathways, indicating a great diversity in proteins involves in the repair mechanism of DSB (58).

5.1.2. HRR in Helicobacter pylori and Mycobacterium tuberculosis: Here we briefly describe the HRR characteristics of these bacteria that make them so resistant to stressful environments.

***5.1.2.1. Helicobacter pylori* case** : The DNA recombination and repair mechanism of the *H. pylori* has been extensively studied, since this bacterium has successfully colonized the human stomach. Hence, it is a very useful microorganism for the understanding of bacteria pathogenicity. The results of an investigation led by Dorer *et al* (61), suggest that *H. pylori* requires the RecA and AddAB proteins for efficient stomach colonization. AddA is required for DSB repair by homologous recombination and the RecA expression is frequently induced by DNA damage, thus increasing induction of SOS (61). Since *H. pylori* is exposed to DNA damage in the stomach, this bacteria requires RecA and AddAB for DNA repair and other recombination events, and in this way it can accomplish the stomach colonization (61).

Genome sequence studies revealed that *H. pylori* lacks LexA, low fidelity polymerases, and a cell cycle repressor, suggesting that *H. pylori* is deficient in the SOS response. An investigation made by Dorer *et al* (61), indicates that individual induction of competence is a key component of the *H. pylori* reaction to DNA damage and implies the existence of a close connection between DNA damage and genetic variability during stomach colonization. Further, Dorer *et al* (61) have demonstrated that genes involved in DNA repair are only one of the many types of genes that are regulated by DNA damage. In response to DNA damage, a variety of genes with different functions are regulated, like the genes required for energy metabolism, membrane protein and fatty acids biosynthesis. But how these genes help the bacteria survival in the case of DNA damage is not well understood (61).

It could be possible that RecA may be necessary for a transcriptional response to DNA damage in *H. pylori*, even though this bacterium seems to lack lexA gene, by means of sensing and transmission of the damage signal. Also Dorer *et al* (61), suggest that under stressful conditions the *H. pylori* strain tested maintains a low mutation rate, which supports the hypothesis proposed by Schwarz and Salama, that *H. pylori* variation is driven by recombination among diverse strains. Further, Dorer *et al* (61) revealed that in *H. pylori* exists a relation between the natural competence and the response to DNA damage (61). An important finding was that in a heterogeneous population of *H. pylori,* a genetic exchange can be induced by signals produced by extreme environments that occasioned DNA damage. The selection of a fitter variation through the re-assortment of pre-existing alleles and the exchange of antibiotic resistance can be increased by the up-regulation of natural competence (61).

In *H. pylori*, has been found two groups of genes that could resolve the branch migration of the Holliday junction, the *ruvABC* and the *recG*. The RecG and RuvB enzymes are helicases, which are a common recombination intermediates and can branch migrate the Holliday junction. The RuvC is an exonuclease, of the RuvABC pathway, that nicks DNA, thus generating the Holliday junction resolution into dsDNA. Studies realized by Kang *et al* (51), try to elucidate which pathway is more prominent in *H. pylori*. These studies revealed that RecG competes with RuvABC for the DNA substrate. Nevertheless, RecG initiates an incomplete pathway in *H. pylori*. Consequently, the Holliday junction cannot be solved in the RecG pathways

causing a failure to repair the replication fork, naturally producing a dead end (51). It has been found that, in *H. pylori* the major recombination repair pathway is the RuvABC, and it is critical for DNA damage repair. Even though the RecG has a role in branch migration, this enzyme interferes with the recombination repair (51).

***5.1.2.2. Mycobacterium tuberculosis* case:** It has been found that during the different stages of the *Mycobacterium* infection, genes from the NER, BER, NHEJ and HRR were expressed; allowing the *Mycobacterium* genome to maintain a great stability. For example, gene expression experiments performed with microarrays suggest that genes involved in the HRR pathway were expressed during the infection active phase (68).

The ability to repair damaged DNA in *M. tuberculosis* is very important in these bacteria because it helps protects them from the immune cells attack. The RuvC enzyme is a specific endonuclease that has a function in the final step of the Holliday junction resolution. The *ruvC* gene is induced following DNA damage (69). It has also been proposed elsewhere that *M. tuberculosis* has at least two mechanisms that control gene expression in response to DNA damage. The first mechanism is mediated by the LexA protein that binds to the SOS box, which is up-stream the regulated gene with liberation of this repression, and requires RecA. The second mechanism is independent of RecA (69).

The *ruvC* gene is part of a group of 28 genes that were identified by genomic analyses and it's thought that it can potentially regulate by both LexA/RecA and alternative mechanisms. The control of the DNA cleavage activity of RuvC is important to facilitate DNA repair (69).

It has also been found in *E. coli* that RuvC is expressed in a very low level and is not induced by DNA damage like in *Mycobacterium*, however its activity is stimulated by interaction with the RuvAB branch migration complex. It was also found that in *E. coli* the RuvC enzyme is controlled post-translationally, and not at the transcriptional level. In the other hand, *ruvC* is transcriptional, regulated with *ruvAB* in *M. tuberculosis* (69).

5.1.3. RecA as a drug target: One of the main problems for the treatment of diseases caused by bacterial pathogen is their ability to become resistant to antibiotics whereby causing an important health problem. As mentioned above, the RecA enzyme plays a very important role in the DNA repair and stalled replication fork, and participates in processes that promote mutations induced by stress and horizontal gene transfer (70).

An important characteristic of the RecA protein is their activation in the presence of ssDNA and ATP. This protein is loaded to the ssDNA with ATP, and forms a helical homopolymeric filament (the RecA-ssDNA filament), which has enzymatic and signaling properties. This ATP molecule is later hydrolyzed (70). This enzyme has been proposed as a bacterial drug target by Sexton *et al* (53), as an adjuvant with the potential to inhibit the mechanism by which bacteria obtain the very harmful drug resistance. The DNA damage repair and stalled replication is the central activity of the RecA protein, but also participates in the adaptive mutagenesis and horizontal gene transfer (53). It has been found that the SOS and recombination processes mediated by RecA protein can be activated in response to antibiotic treatment. Hence, instead of treating diseases we are probably helping the prokaryotic pathogen develop a greater variability, originating a drug resistance strain (53).

Sexton *et al* (53) found that RecA inhibition on bacterial drug resistance is through a high throughput screening that potentially identifies

a RecA inhibitor. The investigators found 40 possible inhibitors of the RecA ATPase activity. Further structured activity relation analysis would be needed for the development of a successful RecA inhibitor (53).

5.2. Non-Homologous end Joining (NHEJ) in prokaryotes: For a long time, NHEJ had not been considered as a mechanism of DNA repair in bacteria. However, NHEJ has recently been identified in bacteria (71). Homologous elements of eukaryotes have been identified in prokaryotes. Some authors accepted that the NHEJ repair pathway is not present in prokarytic and archaeal organisms. However, as we will discuss, this theory has now been refuted by a number of recent studies (72). Current studies have in sighted the role that NEHJ plays in protecting against bacterial DBS of the chromosome via homologous to proteins found in eukaryotes (73,74).

The first evidence for the existence of this pathway in prokarya came from *In silico* studies that identified many bacterial genomes that possess genes encoding putative Ku orthologs. Although the eukaryotic Ku is a heterodimer, the bacterial Ku is usually encoded by a single gene, and biochemical studies have confirmed that the Ku is indeed a homodimer that binds to the termini of DSBs (74). Even if, an amount of bacteria encoded a potential heterodimeric *ku* operonic system suggesting that a gene duplication event occurred early on in the evolution of the NHEJ apparatus. The *ku* genes are often genetically linked in operons with another gene that encodes a putative ATP-dependent DNA ligase (75). Apparently, bacteria possess a few NHEJ-specific genes, including a gene encoding a homodimeric Ku. Generally, the prokaryotic *ku* genes are located in operons containing a conserved ATP-dependent DNA ligase, LigD (ligase D) but the mechanism is not clear.

5.2.1. NHEJ repair mechanisms: A Ku homodimer binds to the ends of the DNA break and recruits LigD. The polymerase domain of LigD binds to a 5´-phosphate (P) and, together with Ku, promotes end-synapsis. The nuclease and polymerase activities of LigD, if necessary, can require other factors to process the break termini to re-establish complementary ends. Finally, ligation of the nick by LigD repairs the break (Fig. 5) (76). Still the biochemical pathways are not clear.

5.2.2. NHEJ in Bacillus subtilis and Mycobacterium sp.: Genes have been identified with homology to Ku70 and Ku80 in some genomes of bacteria, which demonstrates that prokaryotes might have a NHEJ pathway that is homologous to that of eukaryotic cells. Notably, the *ku*-like gene exists in some bacterial species in an operon that includes a gene predicted to encode an ATP-dependent DNA ligase. The operons frequently co-regulate functionally relating protein. These ligases interact with the Ku-like proteins (74). The widespread of the repair mechanism of eukaryotic and prokaryotic NHEJ is dependent on the DNA end-binding protein Ku and a dedicated ATP-dependent DNA ligase (Lig4 in eukarya, LigD in bacteria). Only some sets of bacteria have genes encoding Ku and LigD whereas Ku and Lig4 are present in almost all eukaryal species, among which are the human pathogens *Bacillus* sp., *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* (77).

5.2.2.1. Bacillus subtilis case: DSB are the most critical damage in DNA, caused by ionizing radiation and desiccation in vegetative cells, and are also induced in spores of bacteria. It is important to mention that it requires the participation of two homologous chromosomes for homologous recombination pathway. The spores of *B. subtilis* contain only one chromosome (as toroidal) therefore DSB repair

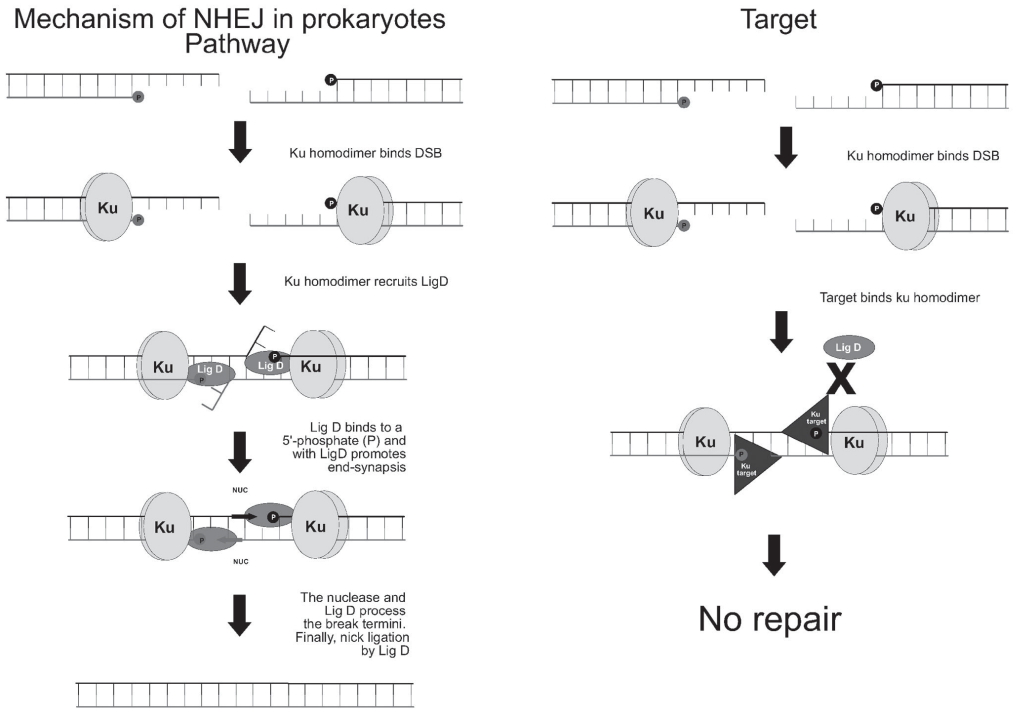

Fig. 5. Signaling pathways in NHEJ repair mechanism prokaryotes with Ku protein and LigD. Possible mechanisms that could be a drug target in the Ku protein. A. Pathway: Ku homodimer binds DBS, then LigD binds to a phosphate 5´ and promotes end-synapsis, which with the nuclease process break termini and finally nick ligation. B. Target: Ku homodimer binds DBS, then Ku target bind to Ku and disrupts repair.

by homologous recombination cannot function during the germination of spores (78).

A different repair pathway to HR for DSB induced in spore DNA, nonhomologous-end joining (NHEJ) has recently been identified in *B. subtilis,* a Ku homolog (encoded by the *ykoV* gene). The bacterial Ku recruits a DNA ligase (encoded by *ykoU*) to DNA ends and in this manner stimulates DNA ligation (78). *Bacillus subtilis* has a heterodimer Ku 70/80 YkoU and a protein with few homologies to ADN ligase IV YkoV.

5.2.2.2. Mycobacterium* sp. *Case: Nonhomologous end-joining (NHEJ) pathway with protein Ku and DNA ligase (LigD) is used by *Mycobacteria* to repair DNA double-strand breaks (DSBs). The mechanisms of mycobacterial NHEJ depend on the structures of the DSBs and end-processing and end-sealing components (77). The Ku like genes are often genetically linked in operons with another gene that encodes an ATP-dependent DNA ligase. It has been also established in *Mycobacterium tuberculosis* that Ku (Mt-Ku) and ligase (Mt-lig) proteins, together reconstitute a mechanism with capacity for ligation (79). Ku in *Mycobacterium tuberculosis* is a homodimer, which binds only to linear DNA ends.

5.2.2.3. E. coli: Other bacteria, where Ku-like and Ligase-D-like proteins have not been found,

are only generally accepted to be end-joining and as the recombination-mediated mechanisms to repair DNA breaks and integrate exogenous sequences. In fact, this bacterium is used as a negative control for experiments in other bacterial NHEJ (80). However, as a strategy for the horizontal transfer of genes in the genome of this bacterium, *E. coli* can integrate unrelated sequences by, non-homologous end-joining. Hence, alternative end-joining (A-EJ) contributes to bacterial genome evolution and adaptation to environmental challenges, but the most interesting fact is that characteristics of A-EJ also come into view in A-NHEJ (80).

5.2.3. Ku protein as a drug target: The key DNA end-binding component of NHEJ is Ku protein. Ku, is a heterodimer of two subunits [Ku70 (69 kD) and Ku80 (83kD)] (*3–5*) present in eukaryotic cells that form a structure through which a diversity of DNA end, has NHEJ has not been reported in prokaryotes. Nevertheless, genes with significant homology to Ku70 and Ku80 have been identified in some bacterial genomes (74).

Several studies have demonstrated that the Ku protein is the homologous most common in bacteria for the NHEJ repair of mechanisms. Here are some examples: the *Bacillus subtilis* gene *ykoV* is adjacent to the *ykoU* gene, which encodes a two domain protein: a catalytic subunit of the eukaryotic-archeal DNA primase (EP) and their juxtaposed with a gene for a eukaryotic-archeal ATP-dependent DNA ligase (ADDL) domains; the YkoV protein is conserved in bacteria that encode an EP. The combination of the *ykoV* and the genes coding for EP or ADDL is maintained in some bacteria. This suggests that these genes belong to the same operon. However, the possible operon position gene arrangement is variable. Therefore, it seems most likely that YkoV form a functional complex with EP, ADDL (75).

These studies insight that prokaryotes might have a NHEJ apparatus that is fundamentally homologous to that of eukaryotic cells. Considerably, the *ku*-like gene exists in some bacterial species in an operon that includes a gene predicted to encode an ATP–dependent DNA ligase. The operons co-regulate the participation of proteins in the same metabolic pathway; this creates the potential for putative ligases to interact with the Ku-like proteins**.** Part of the structure of the Ku protein homologue present in prokaryotes could be targeted for use as drugs and treatments against these pathogens. This protein is highly conserved in bacteria (Fig. 5).

The Ku heterodimer's subunits Ku70 and Ku80 form a dyad-symmetrical molecule with a preformed ring that encircles duplex DNA. Ku does not have interactions with DNA bases and sometimes with the sugar-phosphate (81). This suggests that these structurally support broken DNA ends and that the DNA helix in phase across the junction during end processing and ligation. Moreover it does not interfere with heterodimer present in eukaryotes because homolog proteins are present in prokaryotic homodimers.

Although not previously reported, this mechanism in prokaryotes, could shed us light on repair the damage of double-stranded DNA. One of the most important mechanisms is the NHEJ, which is mainly involved in two components: to find the Ku protein damage (homologous to eukaryotic) and the ligD ligand. NHEJ have been found as highly conserved in bacteria of the genus *Mycobacterium*, *Bacillis subtilis* to be working in operons. More studies are needed to understand this phase.

6. Conclusion

Microorganisms have developed a wide range of DNA repair mechanisms that make them

able to survive the hostile conditions in which they are found when colonizing a host (immune response, production of reactive oxygen species, etc.). These repair mechanisms could be classified mainly by the type of DNA lesion that they fix. For example, for single strand breaks we have the BER and NER pathways whereas, for double strand breaks we have the HRR and NHEJ.

Even though a great deal of studies have been made to gain a better understanding of the repair mechanism in prokaryotes, the new drug discovery studies should focus on inhibition of these mechanisms to avoid the generation of more genetic variation on pathogens. This genetic variation can be translated in the development of multi drug resistant strains, hence generating a serious public health problem.

A critical aspect that should be considered by further studies is what direct consequences will arise from the inhibition of a determined DNA repair mechanism due to its intrinsic characteristics. For example, as we have seen before, BER and NER mechanisms act on single strand breaks to maintain the fidelity of the DNA sequence.

But if we inhibit these mechanisms we could induce errors in DNA replication that in most cases could cause the death of the pathogen but in some cases could lead also to favorable mutations. On the other hand, if we inhibit NHEJ and HRR mechanisms we could surely induce the death of the pathogen without cause any genetic variation because we are leaving a lethal double strand break that could not be repaired.

Another point to consider is to perform more studies related to DNA damage checkpoints. These pathways are the first activated in response to DNA damage producing a cell cycle arrest to allow DNA repair. Recently it has been demonstrated that proteins involved in DNA repair have a role in DNA damage checkpoints (82).

An important aspect is way to develop drugs directed specifically to pathogens that do not affect the microbiota of the gastrointestinal track and oral cavity. The current treatments with nonspecific antibiotics do not discriminate between non-pathogenic and pathogenic organisms within the host (83). The chosen targets should be molecules highly conserved within the groups of pathogen bacteria but with low homology with the molecules of the host. These novel drugs may act as adjuvants of current antibiotics to help delay the development of resistance.

Further studies are highly essential in understanding NHEJ and HRR pathways to develop an effective antimicrobial strategy against pathogens which is of importance for public health.

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