The BER necessities: the repair of DNA damage in human-adapted bacterial pathogens

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Abstract | During colonization and disease, bacterial pathogens must survive the onslaught of the host immune system. A key component of the innate immune response is the generation of reactive oxygen and nitrogen species by phagocytic cells, which target and disrupt pathogen molecules, particularly DNA, and the base excision repair (BER) pathway is the most important mechanism for the repair of such oxidative DNA damage. In this Review, we discuss how the human-specific pathogens *Mycobacterium tuberculosis*, *Helicobacter pylori* and *Neisseria meningitidis* have evolved specialized mechanisms of DNA repair, particularly their BER pathways, compared with model organisms such as *Escherichia coli*. This specialization in DNA repair is likely to reflect the distinct niches occupied by these important human pathogens in the host.

Apurinic or apyrimidinic (AP) sites
Regions of the DNA that lack nucleobases, generally as a result of DNA damage, spontaneous loss or inefficient DNA repair.

Reactive oxygen species
(ROS). Reactive molecules that contain oxygen (such as superoxide, hydrogen peroxide and hydroxyl radicals), which damage macromolecules such as DNA, proteins and lipids.

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All living organisms have evolved mechanisms to repair DNA damage to prevent genome instability and limit the accumulation of deleterious mutations. Genomes are under continual threat from endogenous and exogenous factors that result in aberrant base pairing, DNA crosslinks, modified bases, apurinic or apyrimidinic (AP) sites (also known as abasic sites), single-strand breaks (SSBs), double-strand breaks (DSBs) and the insertion of bulky adducts (BOX 1). These lesions reduce viability by impairing the transcription of essential genes and the stalling of DNA replication; furthermore, the incorporation of incorrect bases can itself be harmful and affect the viability of progeny. Given its fundamental importance, the basis of DNA repair has been extensively studied in both prokaryotes and eukaryotes, particularly in model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae*[1–3]. Owing to the diverse nature of DNA damage, several repair pathways have evolved to rectify this damage and to restore lost genetic information (BOX 2).

Throughout their association with a host, pathogenic microorganisms must survive the immune responses that they trigger during the colonization of mucosal surfaces and following the penetration of the epithelial barrier[4]. Innate immune responses against invading pathogens lead to the production of genotoxic molecules, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are generated by epithelial cells and following phagocytosis of microorganisms by neutrophils and macrophages in a 'respiratory burst' (also known as an 'oxidative burst')[5–8]. Activated neutrophils and macrophages show a rapid increase in oxygen consumption, which is subsequently reduced into superoxide anions (O_2^-) by a membrane-associated NADPH-dependent oxidase and secreted into the phagosome. The importance of this aspect of innate immunity is highlighted by individuals with chronic granulomatous disease (CGD), who are deficient in this NADPH-dependent oxidase. CGD is associated with increased rates of chronic infections caused by bacteria and fungi, including *Staphylococcus aureus* and *Candida albicans*[9]. In the phagosome, superoxide anions are subsequently converted into other more destructive oxygen, nitrogen and chlorine species that cause damage to macromolecules such as DNA, proteins and lipids. For example, superoxide dismutates into hydrogen peroxide and subsequently into hydroxyl radicals ($OH^•$) by the Fenton reaction, or into hypochlorous acid through the action of myeloperoxidase (which is expressed by neutrophils and, to a lesser extent, macrophages[10]); genetic deficiency of myeloperoxidase is associated with susceptibility to certain fungal, but not bacterial, infections[11]. Furthermore, superoxide reacts with the antimicrobial molecule nitric oxide to form the highly reactive oxidant peroxynitrite ($ONOO^-$)[12]. Nitric oxide synthase deficiency has not yet been described in humans, but knockout mice are more prone to bacterial and fungal infections[11], which is also observed

Box 1 | **Causes and consequences of DNA damage in bacteria**

The ability of bacterial pathogens to repair damaged DNA is essential during host colonization and infection, as they are exposed to numerous different DNA damaging agents that cause various types of DNA lesions that require specialized pathways for efficient repair. Depending on the route of transmission, bacterial pathogens can be exposed to ultraviolet radiation and desiccation in aerosol droplets, and to secretions from the host or on environmental surfaces. UV-induced DNA lesions include DNA crosslinks, such as cyclobutane pyrimidine dimers and 6–4 photoproducts[14], both of which create bulges in the DNA that are repaired by the nucleotide excision repair (NER) pathway. Desiccation generally results in intracellular dehydration and double-strand breaks (DSBs) that are repaired by the non-homologous end-joining (NHEJ) or homologous recombination (HR) pathways[105]. Furthermore, during colonization of epithelial cell layers in the nasopharynx, lungs and gastrointestinal tract, and the subsequent infection of deeper tissues, bacteria are exposed to different types of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including hydroxyl radicals, nitric oxide radicals and peroxynitrite, which are produced by epithelial cells or immune cells (such as neutrophils and macrophages). Depending on the specific type of ROS or RNS insult, various forms of DNA damage arise[14] (TABLE 1). The most frequent damage is oxidized and deaminated forms of single nucleobases, such as 7,8-dihydro-8-oxoguanine (8-oxoG) and uracil. The majority of these single-base lesions can be repaired by the base excision repair (BER) pathway; however, lesions that are more bulky (such as pyrimidine dimers) are repaired by the NER pathway, and DSBs are repaired by HR or NHEJ. Furthermore, some bacteria, including *Helicobacter pylori*, colonize the gastrointestinal tract and are exposed to the extreme acidic environment of the gastric juice, as well as to bile in the duodenum and lower parts of the small intestine (TABLE 1). Both environments increase the frequency of DNA damage, and these pathogens require BER, NER and HR for repairing DNA lesions[106–108].

following the administration of nitric oxide synthase inhibitors. Interestingly, knockout mice that lack both NADPH oxidase and nitric oxide synthase show extreme sensitivity to infections (including those caused by *Listeria monocytogenes* and *Salmonella enterica*) and are typically killed by overgrowth of the indigenous microbiota[13], indicating synergistic antimicrobial activity between superoxide and nitric oxide.

The type of DNA lesion caused by these insults varies according to the particular ROS or RNS produced by the host cell and includes SSBs, DSBs and crosslinking of DNA strands[14]. However, the most frequent types of damage are chemical modifications of single DNA bases and the generation of AP sites. Superoxide anions and hydrogen peroxide are relatively unreactive with DNA, but hypochlorous acid is able to chlorinate nucleobases and generally forms 5-chlorocytosine, 8-chloroadenine and 8-chloroguanine[14]. Furthermore, hydroxyl radicals are highly reactive and produce a wide range of oxidized nucleobases, including 7,8-dihydro-8-oxoguanine (8-oxoG), 5-hydroxycytosine, 4,6-diamino-5-formamidopyrimidine (FapyA) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) (reviewed in REF. 14). Nitric oxide can cause DNA damage through nitrosation reactions with primary amines on the bases of DNA, which in downstream reactions lead to deamination[15]. In addition to deamination reactions, peroxynitrite oxidizes DNA and preferentially reacts with guanine to form 8-oxoG[16]. The majority of these chemical modifications and nucleobase lesions are typically resolved by the base excision repair (BER) pathway[17] (FIG. 1).

In general, fundamental processes, such as DNA replication and repair, are highly conserved in bacteria. However, there is increasing evidence that human-adapted bacterial pathogens have evolved specialized pathways of DNA repair, including the BER pathway. It has also become evident that BER facilitates microbial survival in the hostile environment of the host and contributes to virulence. In this Review, we illustrate this emerging concept by comparing the different strategies used by the three bacterial pathogens, *Mycobacterium tuberculosis*, *Helicobacter pylori* and *Neisseria meningitidis* (commonly known as meningococcus), to repair oxidative DNA damage, with an emphasis on the BER pathway. These three pathogens can all successfully colonize a given individual for several months (in the case of meningococcus) or even decades (for *M. tuberculosis* and *H. pylori*), so they exist in an intimate, long-term association with their human host. However, they occupy distinct sites in the body; *N. meningitidis* and *M. tuberculosis* are found in the upper and lower respiratory tract, respectively, whereas *H. pylori* colonizes the gastric mucosa. Furthermore, of these three microorganisms, only *M. tuberculosis* is thought to be predominantly an intracellular pathogen. We outline below how these different lifestyles are reflected in the organization of the BER pathway and the ways in which this pathway interacts with other mechanisms of DNA repair.

The BER pathway

The BER pathway repairs non-distorting DNA lesions following oxidation, deamination or alkylation of bases in the DNA backbone[18]. Initially, a damaged base is recognized and removed by a DNA glycosylase that cleaves the *N*-glycosidic bond between the base and the DNA backbone, generating an AP site (FIG. 1a). Although certain DNA glycosylases are lesion specific, others recognize a wide range of substrates and some share common substrates with other enzymes[17]. Glycosylases can be classified as monofunctional or bifunctional: monofunctional enzymes possess glycosylase activity only, whereas bifunctional enzymes also have lyase activity for cleavage of the DNA backbone at the AP site. Lyase activity can either generate a 3′-α,β-unsaturated aldehyde (through β-elimination) and a 5′-phosphate, or result in a 3′-phosphate (through γ-elimination)[19]. Alternatively, AP sites are cleaved by AP endonucleases, which incise the DNA backbone and leave a 3′-hydroxyl and a 5′-deoxyribose phosphate (dRP)[20]. Subsequently, these products must be processed into 3′-hydroxyl and 5′-phosphate groups before the repair process is complete[19,20]. In short-patch repair, single-nucleotide gaps are filled by the activity of DNA polymerase I (PolA) and are sealed by DNA ligase (LigA). Alternatively, in long-patch repair, PolA replaces up to 10 nucleotides using the 5′-dRP-containing strand as a template[21,22]; the flap is then processed by an endonuclease such as Fen1 before the nick is sealed[23].

The GO system forms part of the BER pathway and specifically prevents oxidation of guanine or repairs DNA containing 8-oxoG and associated mismatches (FIG. 1b). As guanine is one of the most frequent oxidized nucleobases[24], the GO system is particularly important for host-adapted bacteria, as they are continuously exposed to ROS and RNS, both of which generate 8-oxoG. The presence of 8-oxoG can result in the

Reactive nitrogen species
(RNS). Nitrogen-containing oxides, such as peroxynitrite and nitric oxide radicals, which are highly reactive molecules that damage DNA and proteins through oxidation and nitrosation reactions.

Phagocytosis
The mechanism by which phagocytes, such as macrophages and neutrophils, engulf and destroy microorganisms, foreign material and cellular debris.

Respiratory burst
Rapid release of reactive oxygen species and reactive nitrogen species from immune cells, such as macrophages and neutrophils, to attack invading microorganisms. It is initiated by the NADPH-dependent oxidase that converts oxygen into superoxide, which subsequently reacts with other molecules such as nitric oxide to form the highly reactive oxidants peroxynitrite and hydroxyl radials.

Box 2 | **Paradigms of DNA repair in bacteria**

In order to repair DNA damage and prevent loss of genetic information (which is caused by the emergence of mutations), several DNA repair pathways have evolved that have long been considered to operate independently of each other. However, more and more overlap and crosstalk between these pathways have been identified in the past decade. Some repair mechanisms can directly repair specific types of DNA damage by chemical reversion of modified bases , which occurs without disrupting the DNA backbone[109]. DNA damage that is limited to a single strand is repaired by the base excision repair (BER), nucleotide excision repair (NER) or mismatch repair (MMR) pathways. The BER pathway is central to protecting cells from oxidative DNA damage, and from the deamination, alkylation and hydrolysis of bases[17]. Damaged bases are recognized and removed by DNA glycosylases, resulting in an apurinic or apyrimidinic (AP) site[110,111]. The DNA backbone is then incised by a bifunctional glycosylase or AP endonuclease[3,112,113]. Finally, the single-strand gap is replaced with 1 nucleotide (in short-patch BER) or 2–10 nucleotides (in long-patch BER) by a DNA polymerase, and a DNA ligase then seals the gap. The NER pathway recognizes bulky adducts that distort the DNA helix. The UvrABC complex scans the DNA helix for distortions and cleaves the DNA backbone upstream and downstream of the lesion; a DNA helicase then removes the damaged segment[114]. The resulting single-strand gap is filled by a DNA polymerase and is subsequently sealed by DNA ligase. There is another NER pathway, which is coupled to the transcription machinery and enables DNA damage to be efficiently handed over to the NER pathway for repair[115]. This occurs when the NER system recognizes RNA polymerases that are stalled at an abnormal base during transcription. The MMR pathway is involved in repairing mismatched nucleotides and insertions or deletions, which occur during DNA replication or following direct DNA damage. Mismatches are usually recognized by MutS, the newly synthesized strand is incised by MutL (MutH in *Escherichia coli*), and the mismatched base is then removed by the combined action of a DNA helicase, an exonuclease and a single-stranded DNA-binding protein[116]. Repair of double-strand breaks (DSBs) is mediated by the homologous recombination (HR) or non-homologous end-joining (NHEJ) systems[117]. HR is a highly conserved process found in all domains of life[1], whereas the NHEJ pathway is absent in several bacteria, including *E. coli*. The basic steps in HR involve the excision of DNA around the 5′ end of the break, strand invasion by the resulting 3′ overhang, which results in a Holliday junction, followed by cleavage and resolution of this junction[118]. NHEJ directly ligates sequences flanking a DSB, and so it does not involve a homologous template. However, this pathway does not correct a lesion when there has been loss of nucleotides or when the single-stranded DNA overhangs are incompatible[117,119], and can thus lead to mutations. Similarly, the translesion synthesis (TLS) repair pathway can also be mutagenic. In TLS, specialized DNA polymerases continue DNA replication across lesions where the replication fork has stalled[120].

misincorporation of adenine, instead of cytosine, into the DNA backbone. Typically, the GO system consists of two DNA glycosylases, Fapy DNA glycosylase (MutM; also known as Fpg) and MutY, as well as an 8-oxoG triphosphatase (MutT). The DNA glycosylase MutM removes 8-oxoG[25], whereas MutY removes adenines that are base-paired with 8-oxoG to enable the correct insertion of cytosine[26]. MutT hydrolyses intracellular pools of 8-oxoGTP to produce 8-oxoGMP, which reduces the likelihood that 8-oxoGTP is introduced into newly synthesized DNA[27].

The intracellular lifestyle of *M. tuberculosis*

M. tuberculosis, the causative agent of tuberculosis (TB), is one of the most widespread bacterial pathogens and infects one-third of the global population, causing 1.3–1.4 million deaths each year[28]. This bacterium initially colonizes the mucosal surface of the lower respiratory epithelium and is subsequently phagocytized by alveolar macrophages. As one member of a select group of pathogens that survives and replicates in an intracellular environment[29,30], *M. tuberculosis* is primarily found within phagosomes and is able to prevent the maturation of phagosomes into phagolysosomes, which promotes bacterial proliferation[31]. Within these activated macrophages, the bacterium is continuously exposed to ROS, RNS and other antimicrobial factors[32], which control the spread of *M. tuberculosis*. Consistent with this, mice lacking components of enzyme complexes that are responsible for the generation of ROS in phagocytes or missing nitric oxide synthase (which is required for the production of RNS), as well as individuals with CGD who have a defective respiratory burst[33–35], show increased susceptibility to TB. These findings indicate that throughout its life cycle, *M. tuberculosis* is exposed to a variety of DNA damaging agents, and as such, the bacterium has the complete repertoire of DNA repair systems found in other bacteria (with the exception of the mismatch repair (MMR) system) (TABLE 1). The enzymes involved in *M. tuberculosis* DNA repair are regulated by the SOS response through both RecA-dependent and RecA-independent pathways[36,37]. In the RecA-dependent pathway, RecA is activated by DSBs or SSBs and cleaves the repressor LexA, which facilitates the transcription of genes required for DNA repair. The mechanisms underlying the RecA-independent pathway have not been identified, although the binding sites of LexA have been mapped across the genome[38]. However, clear specialization of BER is evident in *M. tuberculosis*, reflecting its intracellular niche and its high GC content, which renders its genome susceptible to oxidation of guanine and deamination of cytosine.

Redundancy in mycobacterial BER. *M. tuberculosis* has evolved remarkable redundancy in its GO system[39,40] (FIG. 2), which repairs 8-oxoG. For example, the bacterium harbours four MutT orthologues (MutT1–MutT4); although MutT1 and MutT2 show high levels of hydrolase activity for 8-oxoGTP, MutT4 exhibits only a low level of hydrolysis[41–43]. Notably, a *mutT1* deletion mutant shows an increase in mutation rate[41], even though MutT1 shares overlapping biochemical activity with MutT2. The multiple MutT orthologues that are encoded by this bacterium emphasize the specialization of BER for dealing with ROS by reducing the cytoplasmic pool of 8-oxoGTP.

Biochemical analyses of the mycobacterial glycosylase MutY have shown that this enzyme has an extended range of substrates compared with the *E. coli* enzyme and can remove adenine, guanine and thymine bases that are mispaired with 8-oxoG[44,45]. Interestingly, *M. tuberculosis* also has two genes that potentially encode MutM glycosylases for removing 8-oxoG from the DNA backbone. The substrate specificity of one of these enzymes, MutM1 formamidopyrimidine-DNA glycosylase 1 (Fpg1), is similar to that of its *E. coli* counterpart[44], whereas the second MutM orthologue, Fpg2, does not bind to DNA, lacks glycosylase activity and seems to be encoded by a pseudogene[46]. In addition, *M. tuberculosis* has two endonuclease VIII (Nei) orthologues that usually excise oxidized pyrimidines, although *E. coli* Nei also excises 8-oxoG mispaired with an adenine or a guanine[47,48]. The *M. tuberculosis* enzymes, Nei1 and

Activated neutrophils and macrophages
Neutrophils and macrophages that undergo morphological changes following triggers (such as cytokines) and that are able to extend pseudopods that assist phagocytosis. In addition, they can rapidly release reactive oxygen species and reactive nitrogen species in a 'respiratory burst', thereby killing engulfed microorganisms.

Superoxide anions
(O_2^-). A common reactive form of oxygen that is generated when molecular oxygen gains an electron. It is a common intermediate in biological processes and is generated by phagocytes to kill microorganisms in a 'respiratory burst'.

Phagosome
Intracellular compartment of a phagocyte that contains phagocytised microorganisms, foreign material or cellular debris.

Chronic granulomatous disease
(CGD). A genetic deficiency of phagocyte oxidase components that is characterized by recurrent infections.

Hydroxyl radicals
($OH^{\bullet}$). Highly reactive oxygen-containing molecules that cause severe damage to macromolecules. They are produced by phagocytes through the conversion of water into superoxide and hydrogen peroxide to kill microorganisms in a 'respiratory burst'.

Fenton reaction
Reaction between hydrogen peroxide and iron salts, mostly via iron–sulfur protein clusters, which generates hydroxyl radicals.

Peroxynitrite
($ONOO^-$). A highly reactive molecule that damages macromolecules, such as DNA and proteins, through oxidation and nitrosation reactions. In phagocytes, it is produced during the 'respiratory burst' through the reaction of superoxide with nitric oxide.

Deamination
Removal of an amine group from nucleobases, amino acids or other molecules.

Alkylation
Transfer of alkyl groups, such as a methyl group or chains with more carbons, between molecules.

β-elimination
Cleavage of the DNA backbone at the 3′ end of an apurinic or apyrimidinic site by a bifunctional DNA glycosylase after removal of a damaged nucleobase. Cleavage produces a 3′-unsaturated aldehyde and a 5′-phosphate group.

γ-elimination
Cleavage of the DNA backbone at the 5′ end of an apurinic or apyrimidinic (AP) site by a bifunctional DNA glycosylase. Cleavage removes the AP site and leaves a phosphate group at both termini.

Nei2, can both excise 8-oxoG when paired with guanine[46,49], albeit at low levels. Given this inefficiency, the Nei enzymes probably function in conjunction with the endonuclease Nth, which is thought to remove 8-oxoG from the DNA backbone during replication[50]. Nei1 also excises uracil from the DNA backbone[46], which arises through the deamination of cytosine.

In addition, the high GC content of the genome may make the bacterium particularly susceptible to deamination of cytosine, which results in its conversion into uracil; alternatively, efficient repair of deamination might be responsible for the high GC content of the genome. Incorporation of uracil into the genome is minimized by the presence of the mycobacterial dUTPase, which hydrolyses dUTP and thereby reduces the intracellular pool[51]; interestingly, dUTPase also deaminates dCTP[52]. In *E. coli*, uracil is excised from the genome by the glycosylase Ung (also known as Udg), which is a component of BER[53,54]. Similarly, mycobacterial Ung efficiently excises uracil from both single- and double-stranded DNA[55]. Notably, Ung is required for full virulence of *M. tuberculosis* in a murine model of infection[56], and for the growth of non-pathogenic *Mycobacterium smegmatis* in murine macrophages[57]. In addition to Ung, *M. tuberculosis* contains a second uracil DNA glycosylase, UdgB[58], which recognizes a broad range of substrates, including hypoxanthine and ethenocytosine[59]. Furthermore, UdgB has the unusual property of being able to bind to AP sites, which are cytotoxic and mutagenic; thus, it has been suggested that UdgB might shield AP sites until the downstream BER enzymes process these lesions[40,58].

Inside phagosomes, bacteria are also exposed to DNA alkylating agents that arise from nitrosation reactions between nitrous anhydride and amines or amides[60]. Both *E. coli* and *M. tuberculosis* contain an O^6-alkylguanine DNA alkyltransferase (known as Ada and Ogt, respectively), which reverses the O^6-alkylation of guanine[61]. In *E. coli*, alkylated bases can also be repaired by the 3-methyladenine DNA glycosylases, TagA and AlkA, which are part of the BER pathway. Although TagA is a highly specific enzyme that only excises 3-methyladenine and 3-methylguanine, AlkA recognizes a wide range of alkylated bases[62]. Interestingly, in *M. tuberculosis* the genes *ada* and *alkA* are found in the same operon as *ogt,* and *ada* and *alkA* encode a fusion protein[60,63]. Mutants lacking this operon are sensitive to alkylating agents but do not show virulence attenuation in mice[60]. Consistent with this, *M. tuberculosis* has two other putative 3-methyladenine DNA glycosylases, TagA and Mpg[63], which could provide functional redundancy for repairing damage following nucleobase alkylation[39].

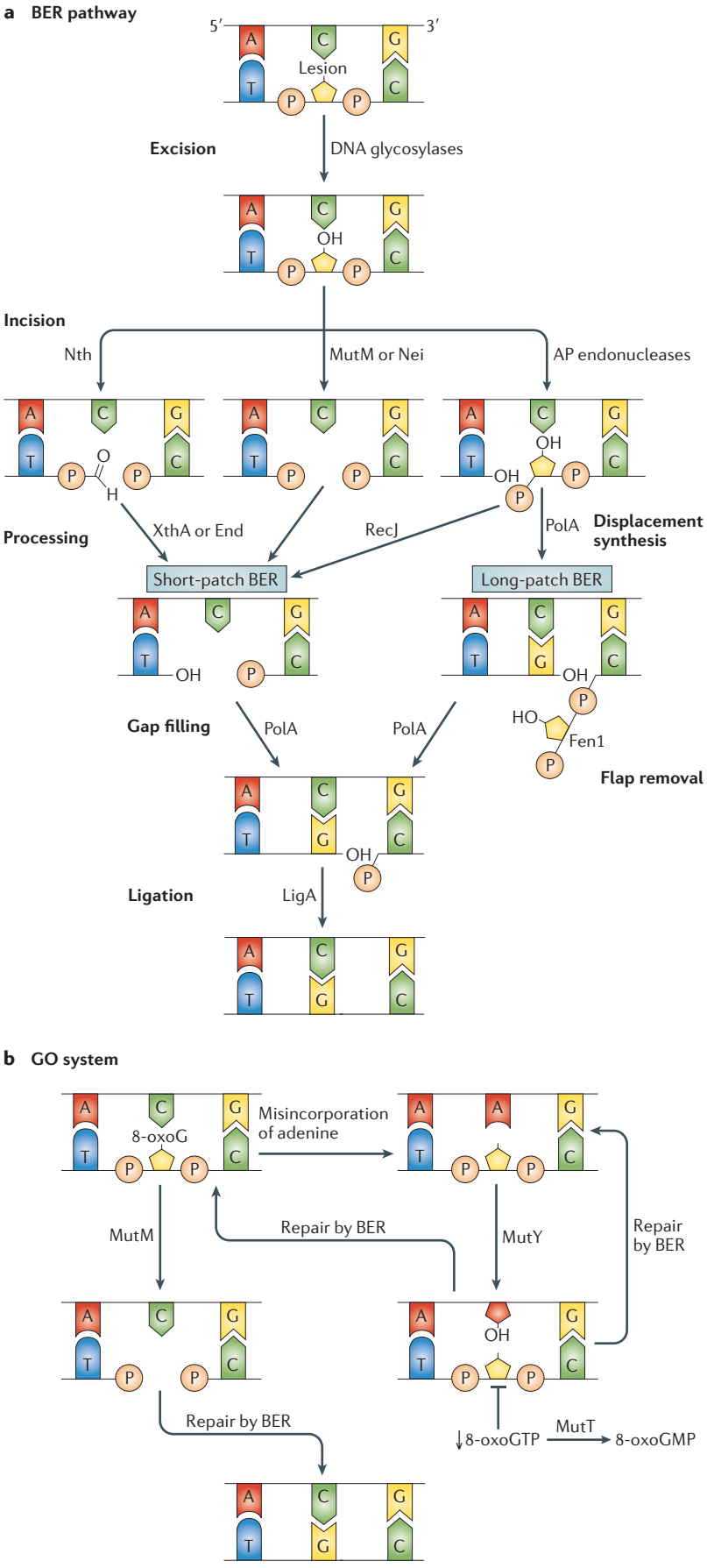
AP sites can result in mutation or stalling of the replication fork[64]. In *E. coli,* AP sites are repaired by endonucleases belonging to the exonuclease III (XthA) and endonuclease IV (End; also known as Nfo) families. Similarly, homologues of XthA and End have been identified in *M. tuberculosis*[63]. However, in *E. coli*, XthA contributes 90% of the AP endonuclease activity, whereas in *M. tuberculosis*, End is the major AP endonuclease and XthA functions predominantly as a 3′-to-5′ exonuclease[65]. Interestingly, both of the *M. tuberculosis* AP endonucleases preferentially process AP sites that form opposite cytosine residues, whereas the *E. coli* AP endonucleases do not exhibit a substrate preference[65,66]. The additional bias of the *M. tuberculosis* BER pathway towards the repair of 8-oxoG and associated mismatches (those that involve mispairing with adenine primarily) further emphasizes how the bacterium has adapted to resist oxidative stress and to counteract the risks associated with a high GC content. Consistent with this, *M. tuberculosis* mutants lacking End and XthA display increased sensitivity to oxidative stress[65], and an *xthA* mutant also has attenuated virulence in mice[56].

Figure 1 | **Schematic representation of the BER pathway. a** | Damaged bases in DNA are recognized by DNA glycosylases (such as formamidopyrimidine DNA glycosylase (MutM), Nth, Nei, MutY, Ung, AlkA, Mug, and Tag) and are removed. During excision, the damaged base is removed via the action of DNA glycosylases. Bifunctional glycosylases also cleave the DNA backbone (termed incision) after base excision; this occurs through β-elimination (by Nth) or δ-elimination (by MutM or Nei). Apurinic/apyrimidinic (AP) endonucleases (XthA or members of the End family) also cleave the DNA backbone during base excision repair (BER). In short-patch BER, the termini generated by cleavage are processed by an AP endonuclease (XthA or an End enzyme), a single-stranded DNA-specific exonuclease (RecJ) or a δ-eliminating DNA glycosylase (MutM or Nei), resulting in 5′-phosphate and 3′-hydroxyl groups. The gap is filled by DNA polymerase I (PolA), and the ends are ligated by LigA. In long-patch BER, 2–10 nucleotides are added by PolA (in a process known as displacement synthesis), and the resulting flap is removed by Fen1; the ends are then ligated by LigA. **b** | The GO system specifically recognizes and repairs the oxidized form of guanine, 7,8-dihydro-8-oxoguanine (8-oxoG). This base is recognized and removed by the DNA glycosylase MutM, and the resulting AP site is repaired by BER. When adenine is misincorporated (instead of cytosine) opposite an 8-oxoG, the DNA glycosylase MutY excises the adenine, and the AP site is subsequently repaired by BER, leading to the correct incorporation of a cytosine or another misincorporation of an adenine. To prevent the misincorporation of 8-oxoG, the 8-oxoG triphosphatase MutT hydrolyses 8-oxoGTP into 8-oxoGMP.

In conclusion, as an intracellular bacterium, *M. tuberculosis* is continuously exposed to ROS and RNS and, as the majority of the damage caused by these oxidants is single-nucleobase lesions, *M. tuberculosis* has evolved extraordinary redundancy in its BER pathway. This functional redundancy is demonstrated by the presence of no fewer than four *mutT* orthologues, two *nei* orthologues, two *mutM* orthologues and two *ung* orthologues, which highlights the specialization of *M. tuberculosis* in its ability to repair oxidative DNA damage.

Surviving in the gastric mucosa: *H. pylori*

H. pylori colonizes the gastric mucosa in approximately 50% of the global population and causes chronic gastroduodenal disorders such as gastric cancer, peptic ulcers and gastritis[67]. The precise mode of transmission of *H. pylori* remains unknown, but it has been suggested



that direct oral–oral or faecal–oral transmission are the most probable modes[68]. *H. pylori* has been detected in saliva, vomit and faeces; the bacterium is highly sensitive to conditions outside the body, such as atmospheric oxygen pressure and temperatures below 34 °C[67]. After penetration of the gastric mucus, *H. pylori* resides in close proximity to the gastric epithelial layer and elicits innate immune responses, resulting in subsequent exposure to ROS and RNS produced by epithelial cells and recruited phagocytic cells, such as neutrophils[69–71] (FIG. 3; TABLE 1). To survive these insults, *H. pylori* must detect and repair DNA damage; however, little is known about the regulation of enzymes required for DNA repair. There is surprisingly limited redundancy in DNA repair pathways, including BER, and the bacterium also lacks functional MMR and non-homologous end-joining (NHEJ) pathways[72,73].

Functional specialization of BER. *H. pylori* contains the minimal complement of enzymes needed to carry out each step of the BER pathway, all of which are necessary to repair single-base lesions caused by ROS and RNS (FIG. 3). Interestingly, the BER components of *H. pylori* seem to have higher specificity for cytotoxic base lesions than mutagenic base lesions. This specialization might contribute to the high genetic variability that is observed among *H. pylori* strains and between individual bacteria during long-term colonization. It has been suggested that this high genetic variability, combined with a high mutation rate, provides *H. pylori* with an adaptive advantage in the gastric environment of the human host[67]. A high mutation rate, owing to the reduced proofreading capacity of its DNA polymerase[74], may increase the chance of *H. pylori* acquiring mutations that confer a fitness benefit under stressful conditions, despite the inherent cost of deleterious mutations. As there is little competition with other microorganisms in the gastric environment, loss of a fraction of the population due to deleterious mutations might not be a major drawback for *H. pylori*, and the high mutation rate might therefore be more beneficial for *H. pylori* than for pathogens that inhabit crowded sites of the body. Consistent with this, a study of human volunteers and primates challenged with *H. pylori* indicated that the bacterium has an increased mutation rate on exposure to the host inflammatory response[75]. Thus, this might increase the emergence of genetic variants of *H. pylori* that could survive the selective pressure imposed by the innate immune response of the host.

H. pylori contains a single DNA glycosylase, Nth, for the removal of oxidized nucleobases. Nth is a bifunctional glycosylase that initially excises oxidized pyrimidines and subsequently excises the AP site. Although oxidized purines (such as 8-oxoG) are far more abundant than oxidized pyrimidines, the former tend to be mostly mutagenic. By contrast, oxidized pyrimidines have the propensity to block DNA replication and transcription; therefore, they tend to be cytotoxic[76]. Consistent with this, *H. pylori* strains lacking Nth are sensitive to oxidizing agents, are preferentially killed by activated macrophages and are impaired in their ability

Table 1 | **Niches occupied by *M. tuberculosis*, *H. pylori* and *N. meningitidis* and related stresses**

Niche	Stresses	Main types of DNA damage
Mycobacterium tuberculosis		
Respiratory droplets	UV radiation and desiccation	DNA crosslinks, photoproducts, bulky adducts and DSBs
Respiratory epithelium	ROS and RNS	Single-base lesions
Intracellular (inside macrophages)	ROS and RNS	Single-base lesions
Granuloma	Hypoxia, ROS, RNS and starvation	DSBs, single-base lesions and possibly other lesions
Helicobacter pylori		
Gastric juice	Low pH	DSBs, bulky adducts and single-base lesions
Gastric mucosal epithelium	Low pH, ROS and RNS	Single-base lesions, DSBs and bulky adducts
Intracellular (inside neutrophils)	ROS and RNS	Single-base lesions
Upper intestine (duodenum)	Low pH and bile	DSBs, bulky adducts and single-base lesions
Neisseria meningitidis		
Respiratory droplets	UV radiation and desiccation	DNA crosslinks, photoproducts, bulky adducts and DSBs
Nasopharyngeal epithelium	ROS and RNS	Single-base lesions
Intracellular (inside neutrophils)	ROS and RNS	Single-base lesions
Blood	Iron starvation and low oxygen	Unknown
Cerebrospinal fluid (which contains neutrophils)	ROS and RNS	Single-base lesions

BER, base excision repair; DSB, double-strand break; HR, homologous recombination; NER, nucleotide excision repair; NHEJ, non-homologous end-joining; RNS, reactive nitrogen species; ROS, reactive oxygen species; UV, ultraviolet.

SOS response
An inducible pathway in bacteria that is activated on the accumulation of single-stranded DNA as a result of DNA damage or the collapse of replication forks. This response typically involves DNA repair proteins and translesion DNA polymerases.

UvrABC complex
A multicomponent enzyme complex that has endonucleolytic activity and is involved in the nucleotide excision repair pathway.

Holliday junction
An assembly of four DNA strands that forms during certain types of genetic recombination.

Hypoxanthine
A deamination product of adenine that is highly mutagenic owing to its ability to base-pair with cytosine and adenine during replication.

Ethenocytosine
A highly mutagenic adduct that is formed by the alkylation of cytosine.

Phase variation
A heritable change in the level of expression of a protein. It often occurs through an alteration in repetitive DNA sequences.

to colonize or persist in a mouse model of infection[76]. At the level of recognition and excision of damaged bases, BER is different in *E. coli*, as this organism contains the DNA glycosylases MutM and Nei, which provide some redundancy for removing oxidized nucleobases. A similar lack of redundancy is found in the ability of *H. pylori* to remove methylated nucleobases from the DNA backbone. *E. coli* contains two 3-methyladenine glycosylases, TagA and AlkA, whereas *H. pylori* contains a single inducible, monofunctional 3-methyladenine DNA glycosylase (MagIII) that belongs to a different enzyme class[77,78]. MagIII contains structural motifs found in AlkA DNA glycosylases, but its substrates are similar to those recognized by *E. coli* TagA. MagIII is highly specific for 3-methyladenines owing to the structure of its binding pocket, which excludes 7-methylguanine and other methylated bases[78]. 3-Methyladenines are particularly harmful because they block DNA synthesis, whereas 7-methylguanine is mostly mutagenic[78]. Furthermore, MagIII is required for survival during alkylation stress and for gastric colonization[77,79]. In addition, *H. pylori* has a potential uracil DNA glycosylase Ung; although this enzyme has not been characterized in detail, it is required for gastric colonization[79]. Thus, at the level of DNA glycosylases, there seems to be little redundancy in *H. pylori* BER.

Other features of the minimal BER pathway found in *H. pylori* include its single AP endonuclease[73,80,81] and the absence of the GO system, although the organism does encode the glycosylase MutY, which excises bases mismatched with 8-oxoG. Specifically, MutY excises adenines that are base-paired with 8-oxoG but, unlike *E.coli* MutY, it does not excise adenines that are paired with guanine[80,82]. Despite this limited range of activity, MutY reduces the level of spontaneous mutations in *H. pylori*, which is evident from the increased mutation rate of a *mutY* deletion mutant[80,81]. Furthermore, MutY contributes to gastric colonization of *H. pylori*[82]. Interestingly, *mutY* contains a homopolymeric tract of eight adenines[81], and slipped-strand mispairing during DNA replication can lead to frameshift mutations, which leads to the production of non-functional MutY and a mutator phenotype. This highlights how *H. pylori* maintains a delicate balance between DNA mutagenesis and repair: MutY phase variation results in a subpopulation of *H. pylori* with an increased mutation rate and potentially a higher propensity of acquiring adaptive mutations. Furthermore, *H. pylori* does not contain an intact MMR system, even though it does express a MutS2 homologue[83]. However, this enzyme inhibits homologous recombination[83], has high affinity for double-stranded DNA that contains 8-oxoG and protects cells against oxidative stress through unknown mechanisms[84].

In summary, *H. pylori* is found in the gastric mucosa in close association with epithelial cells and infiltrating neutrophils, and is subsequently exposed to a strong respiratory burst. *H. pylori* has evolved a highly specialized BER pathway to repair the resulting oxidative DNA damage. The BER components of this pathogen show high specificity for nucleobase lesions that are cytotoxic,

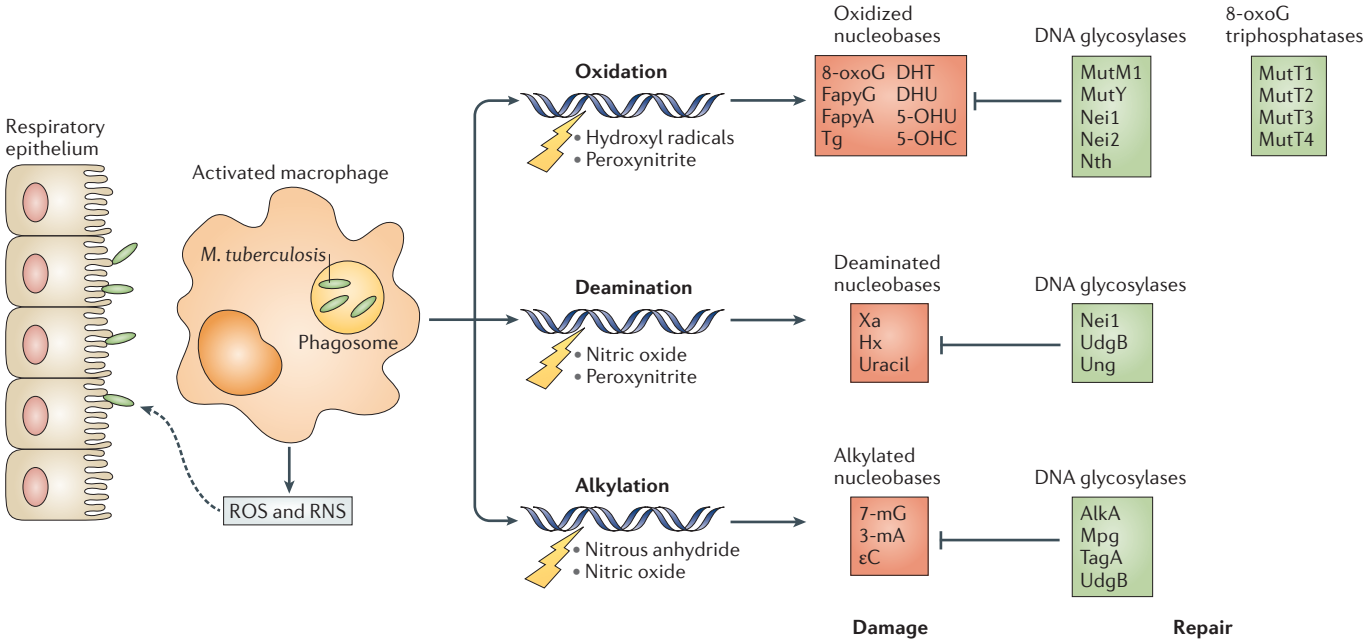


Figure 2 | **Redundancy in the *Mycobacterium tuberculosis* BER pathway.** *M. tuberculosis* colonizes the respiratory epithelium and is subsequently phagocytized by infiltrating alveolar macrophages and other phagocytic cells. Inside the phagosome of activated macrophages, the bacterium is exposed to reactive oxygen species (ROS) and reactive nitrogen species (RNS), resulting in many modifications of nucleobases through oxidation (via hydroxyl radicals and peroxynitrite), deamination (via nitric oxide and peroxynitrite) and alkylation (via nitrous anhydride and nitric oxide) reactions. The most frequently encountered oxidized nucleobases include 7,8-dihydro-8-oxoguanine (8-oxoG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 4,6-diamino-5-formamidopyrimidine (FapyA), thymine glycol (Tg), 5,6-dihydrothymine (DHT), 5,6-dihydrouracil (DHU), 5-hydroxyuracil (5-OHU) and 5-hydroxycytosine (5-OHC). The most common deaminated nucleobases include xanthine (Xa), hypoxanthine (Hx) and uracil, and the most frequent alkylated nucleobases are 7-methylguanine (7-mG), 3-methyladenine (3-mA) and ethenocytosine (εC). These single-nucleotide lesions are generally repaired by base excision repair (BER), and *M. tuberculosis* has evolved remarkable functional redundancy in this pathway. It expresses a large number of DNA glycosylases that recognize and excise a wide variety of damaged nucleobases. For example, *M. tuberculosis* expresses MutY, which excises adenine, guanine and thymine following their mispairing with 8-oxoG. *M. tuberculosis* also harbours four genes encoding potential 8-oxoG triphosphatase (MutT) orthologues, and it expresses the two apurinic or apyrimidinic (AP) endonucleases XthA and End for the repair of AP sites (not shown).

whereas mutagenic lesions are generally untargeted. This specialization might be related to the high genetic variability and mutation rate that are typically observed in *H. pylori* strains[67].

Networks of DNA repair in *N. meningitidis*

The natural habitat of *N. meningitidis* is the upper respiratory tract[85,86] where the bacterium causes asymptomatic infection in 10–40% of healthy individuals[85,87], and carriage of a single strain can last for several months. During infection, the bacterium crosses the epithelial barrier in the upper respiratory tract and enters the bloodstream, resulting in septicaemia and spread to the meningeal space. In contrast to *M. tuberculosis* and *H. pylori*, meningococcus is exposed to atmospheric oxygen concentrations during colonization of the mucosal surface of the nasopharynx during its life cycle. Therefore, this bacterium is exposed to substantial oxidative stress owing to the high extracellular oxygen concentration and its presence inside immune cells (including neutrophils) that generate ROS and RNS[88]. Consistent with this constant exposure to oxidative stress, enzymes involved in BER are constitutively expressed[89,90], rather than being under the control of the SOS response (as in *E. coli*)[91]. Furthermore, similarly to its close relative the gonococcus, *N. meningitidis* can survive within the hostile environment of neutrophils[88], which is thought to promote a rapidly progressive and severe disease, leading to extremely high bacterial densities in the bloodstream. The adaptation of the bacterium to the upper airways and its interactions with immune cells have probably provided selective pressure for the evolution of a distinct BER pathway in *N. meningitidis* compared with model organisms such as *E. coli*[92] (FIG. 4).

Specialization and integration of meningococcal BER. The initial recognition and processing of DNA lesions in meningococcus involve the overlapping activity of DNA glycosylases[90], as shown by the redundancy of the bifunctional glycosylases, MutM and Nth[90,93]. These enzymes recognize and process a variety of DNA lesions: both enzymes eliminate 8-oxoG, 5′-OH substituted uracil (5′-OHU) and thymine glycol (Tg) from the DNA backbone and have similar levels of activity. However, unlike Nth, MutM activity is inhibited by the presence of its products, including the incised DNA backbone with a 3′-phosphate, which reduces the processivity of this

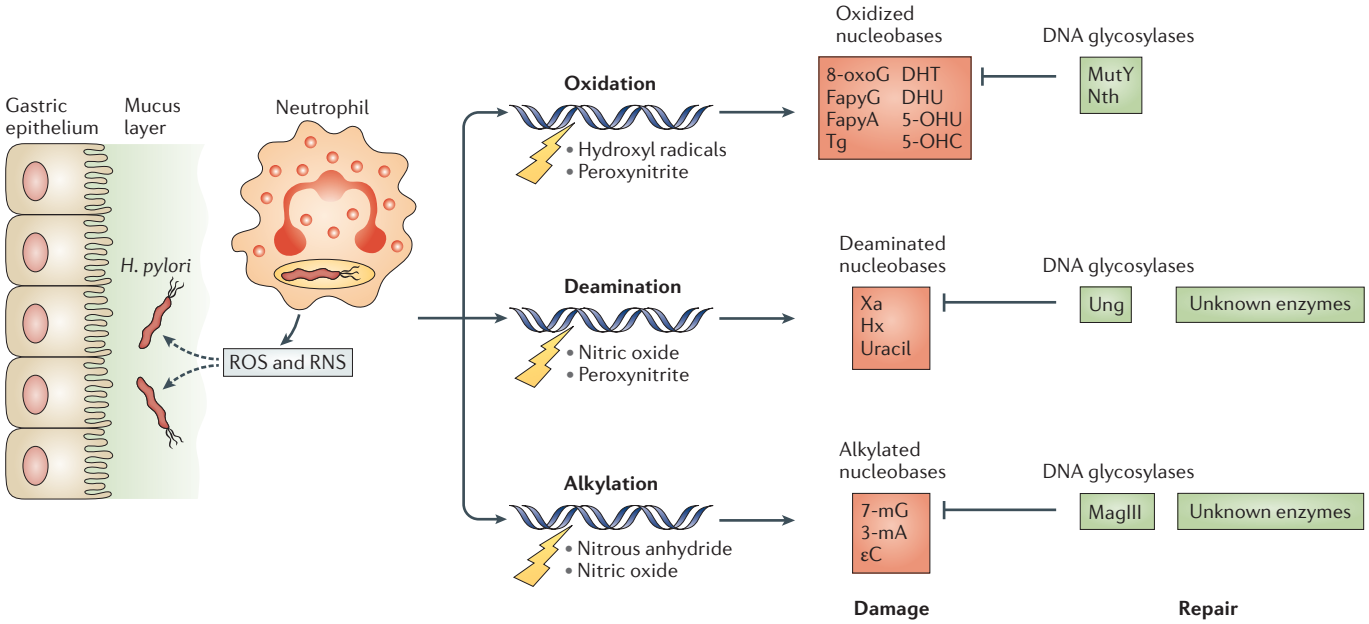


Figure 3 | **Functional specialization of the *Helicobacter pylori* BER pathway.** *H. pylori* colonizes the gastric mucosa and can also be phagocytized by infiltrating neutrophils. Both intracellular and extracellular *H. pylori* are exposed to reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are generated by a strong 'respiratory burst' from neutrophils. ROS and RNS damage DNA by oxidation reactions (mediated by hydroxyl radicals and peroxynitrite), which generate 7,8-dihydro-8-oxoguanine (8-oxoG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 4,6-diamino-5-formamidopyrimidine (FapyA), thymine glycol (Tg), 5,6-dihydrothymine (DHT), 5,6-dihydrouracil (DHU), 5-hydroxyuracil (5-OHU) and 5-hydroxycytosine (5-OHC); deamination (mediated by nitric oxide and peroxynitrite), which generates xanthine (Xa), hypoxanthine (Hx) and uracil; and alkylation (mediated by nitrous anhydride and nitric oxide), which generates 7-methylguanine (7-mG), 3-methyladenine (3-mA) and ethenocytosine (εC). These single-nucleobase lesions are repaired by the base excision repair (BER) pathway. *H. pylori* has three DNA glycosylases (Nth, Ung and MagIII) that recognize and excise several of the most cytotoxic nucleobases. Interestingly, these DNA glycosylases do not recognize and excise nucleobase lesions that are mutagenic. However, *H. pylori* MutY excises adenine mispaired with 8-oxoG and thereby reduces the level of spontaneous mutations. Furthermore, *H. pylori* has the apurinic or apyrimidinic (AP) endonuclease XthA for the repair of AP sites (not shown).

enzyme[90]. Therefore, Nth is more effective than MutM in conditions of higher oxidative stress and DNA damage, such as during exposure to a respiratory burst inside neutrophils or in the aerobic conditions of the upper respiratory tract.

By contrast, there is exquisite specialization of the enzymes involved in subsequent steps of the repair process. Meningococcus has two AP endonuclease homologues, NApe and NExo, which show a high level of structural homology[89]; both of these enzymes contribute to bacterial survival during systemic disease and inside neutrophils[89]. Despite their structural similarity, NApe is the sole active AP endonuclease in meningococcus, whereas NExo is unable to cleave AP sites. Instead, NExo plays a part in BER through its 3′-phosphatase activity[94], which contributes to the repair of residual lesions, the 3′-phosphate and 3′-unsaturated aldehyde, following the lyase activity of MutM or spontaneous base loss through direct DNA damage. Remarkably, this specialization of activity is altered by a single amino acid substitution in each enzyme; substitution of His167 in NExo with either Gly or Ser (which are found in other known AP endonucleases, such as those in *E. coli*) confers AP endonuclease activity on NExo, and introducing His167 into NApe is sufficient for it to function as a 5′-phosphatase[94]. By contrast, both of these activities are carried out by a single enzyme, ExoIII, in *E. coli*[95]. Therefore, the meningococcal processing enzymes NApe and NExo have a crucial role in bacterial survival in the presence of oxidative stress because the groups that they recognize (3′-phosphate, which is recognized by NExo, and 5′-dRP, which is recognized by both) block DNA replication and are therefore cytotoxic.

It has been suggested that potentially cytotoxic intermediates of BER are passed from one enzyme to the next in a defined series of steps during DNA repair. However, there seems to be little integration between the initial and subsequent stages of BER in meningococcus. For example, functional studies of the glycosylases Nth and MutM have shown that the presence of a processing enzyme (such as NApe or NExo) does not promote their activity and processing of the lesion[90]. Thus, BER in meningococcus does not seem to be arranged as an ordered pathway, as proposed for other bacteria, in which the products of the repair process are passed in a sequential manner from one enzyme to the next (FIG. 1). Instead, a collection of constitutively expressed glycosylases, including MutM and Nth, with overlapping activities identify and excise the damaged bases, and the subsequent steps of repair are not determined by complexes of cooperating

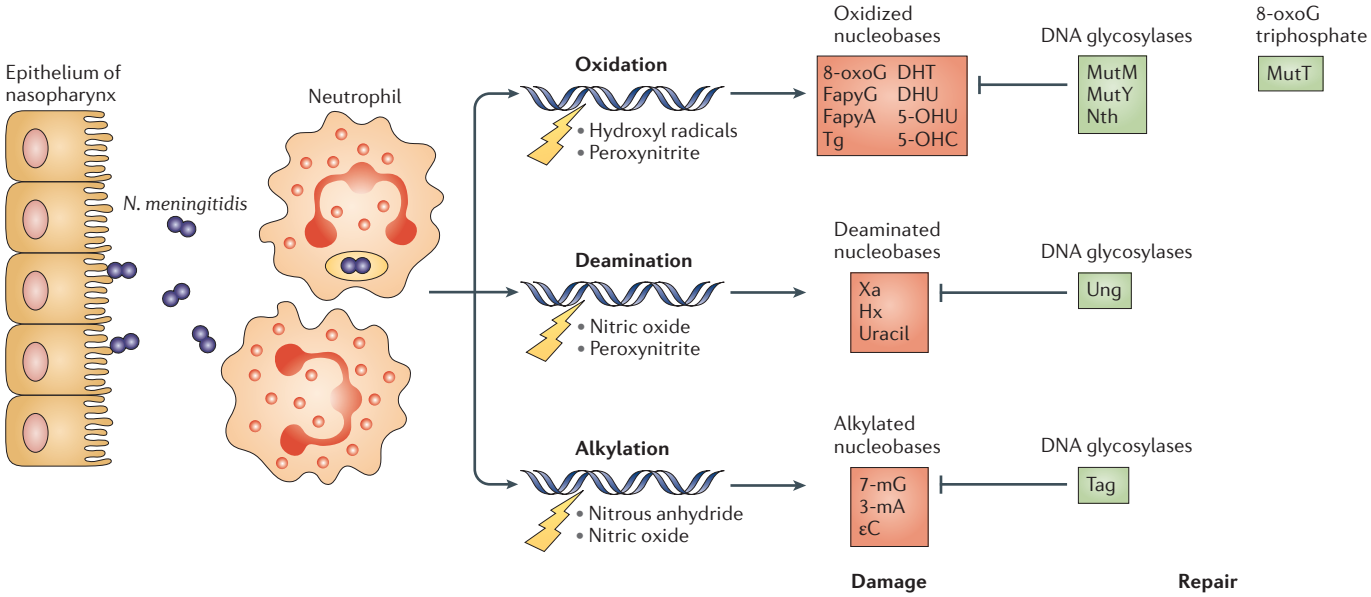


Figure 4 | **The BER network of *Neisseria meningitidis*.** *N. meningitidis* colonizes the human nasopharynx, where it is exposed to reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by atmospheric oxygen and by the 'respiratory burst' of phagocytes. Oxidized nucleobases include 7,8-dihydro-8-oxoguanine (8-oxoG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 4,6-diamino-5-formamidopyrimidine (FapyA), thymine glycol (Tg), 5,6-dihydrothymine (DHT), 5,6-dihydrouracil (DHU), 5-hydroxyuracil (5-OHU) and 5-hydroxycytosine (5-OHC). Deaminated bases include xanthine (Xa), hypoxanthine (Hx) and uracil; alkylated nucleobases include 7-methylguanine (7-mG), 3-methyladenine (3-mA) and ethenocytosine (εC). *N. meningitidis* has several DNA glycosylases with overlapping functions (such as MutM and Nth) that excise oxidized nucleobases. *N. meningitidis* harbours a putative uracil DNA glycosylase (Ung) for the removal of uracil (it is unknown whether this enzyme also removes the other deaminated bases Xa and Hx) and a putative 3-methyladenine DNA glycosylase (Tag) that removes 3-methyladenine (it is unknown whether this enzyme also removes the other alkylated bases 7-mG and εC). In addition, *N. meningitidis* MutY excises adenine, guanine and thymine mispaired with 8-oxoG; the bacterium contains a single 8-oxoG triphosphatase (MutT) homologue, which hydrolyses cellular 8-oxoGTP to 8-oxoGMP. Finally, meningococcal NApe is a true apurinic/apyrimidinic (AP) endonuclease, whereas NExo lacks AP endonuclease activity but instead is a specialized 3′-phosphatase (not shown).

enzymes but by the activities and abundance of the processing enzymes. Indeed, consistent with this, there is evidence that both MMR and NER contribute to meningococcal survival following oxidative DNA damage[90,96]. Thus, meningococcus integrates multiple enzymes and mechanisms of DNA repair, which are organized as a network rather than a pathway, to restore genomic integrity following oxidative DNA damage.

GO or stop during DNA repair? *N. meningitidis* contains a rudimentary GO system that consists of the glycosylases MutM and MutY, as well as a single MutT homologue[90,97]. A meningococcal strain lacking all three enzymes is viable and is not hypersensitive to oxidizing agents, so the GO system does not seem to be a major contributor to the repair of oxidative DNA damage. By contrast, in other bacteria, such as *Pseudomonas aeruginosa,* loss of MutY, MutT or MutM impairs bacterial survival *in vivo* and confers hypersensitivity to ROS[98]. By removing 8-oxoG and reducing the misincorporation of bases, the GO system in meningococcus limits the emergence of spontaneous point mutations, which are detected by the appearance of spontaneous streptomycin-resistant colonies (owing to amino acid substitutions in the ribosomal protein RpsL). As these changes do not affect DNA replication, loss of the GO system does not impair survival under conditions of oxidative stress, but it does increase mutation rate. By contrast, enzymes that process intermediate products in BER (such as NApe and NExo) and that can block replication when they are absent, have major effects on bacterial viability but have only a minor effect on mutation rate[90]. The meningococcal genome also contains genes encoding putative enzymes, Ung and Tag, which excise deaminated and alkylated bases, respectively.

Finally, NER also seems to contribute to the repair of oxidative DNA damage in meningococcus, which is consistent with observations in gonococcus on exposure to low levels of oxidizing agents[99]. However, the loss of NER seems to have a more detrimental effect on the survival of meningococcus than gonococcus in the presence of oxidizing agents[90]. Given the role of NER in repairing damage caused by ultraviolet radiation, this difference might reflect the different body sites at which these bacteria are found in the human host, with meningococcus (in the respiratory tract) being more likely to be directly exposed to ultraviolet radiation than gonococcus, which is found in the genital tract.

Conclusions and future prospects

In the past, the majority of research on pathogenic bacteria has focused on their ability to invade the host and

promote virulence. However, as we learn more about how microorganisms are adapted to particular microenvironments during the different stages of the disease process, it is becoming clear that activities that were once considered 'housekeeping' are also specialized and fit for purpose. This is evident even when considering a highly conserved process such as BER, which is found in humans and prokaryotes. Even between the three bacterial pathogens considered here, there is substantial variation in the complement and function of BER enzymes, which reflects the diversity of habitats that they inhabit in the human body. Furthermore, some accepted paradigms of DNA repair do not hold for these organisms, which can survive in close association with a hostile host. For example, a RecA-dependent SOS response, which has a crucial role in the induction of DNA repair in *E. coli*, is not found in *N. meningitidis*, and a RecA-independent pathway of induction is found in *M. tuberculosis*. Little is known about the regulation of DNA repair in *H. pylori*, except that RecA is subject to post-translational modification[100] and that it is constitutively expressed[101]. Further studies on the regulation of enzymes in BER in these bacteria might reveal the environmental cues that trigger gene expression. Furthermore, DNA repair pathways are thought to operate independently of each other in *E. coli*. The extent of interaction between BER and NER in *N. meningitidis* suggests that future work will uncover other examples of integration of repair pathways in pathogens.

Distinctive mechanisms of DNA repair may well have evolved in other human-adapted microorganisms, including commensal species and other pathogens. For example, bioinformatic analyses have shown that the commensal species *Neisseria lactamica* and *Neisseria polysaccharea* have the same repertoire of BER enzymes as meningococcus (S. v. d. V. and C. M. T., unpublished observations), so their evolution is likely to have been shaped by survival at the mucosal surface. However, these enzymes have several polymorphisms and their activities are poorly understood, so future work in the field should determine whether these polymorphisms affect enzyme function and whether their regulation is altered compared to pathogens. This should provide insights into whether DNA repair is specific for the pathogenic lifestyle or reflects general adaptations that both commensals and pathogens have evolved to enable colonization and transmission. Furthermore, *Streptococcus pneumoniae* can secrete hydrogen peroxide, which modulates host inflammatory responses[102]; the bacterium withstands this genotoxic agent without having a catalase enzyme. Although *S. pneumoniae* has other mechanisms to eliminate ROS, little is known about its ability to correct DNA damage through BER. *Haemophilus influenzae* is another pathogen found in the nasopharynx, and comparative genome analysis indicates that it has a similar complement of BER enzymes as meningococcus[103]. Therefore, further studies on *H. influenzae* have the potential to demonstrate whether the network model of DNA repair is a more general phenomenom or whether it is restricted to *N. meningitidis*.

Several technical challenges remain. Appropriate animal models to study the behaviour of human-adapted pathogens in physiologically relevant settings are lacking, and the construction of bacterial strains with multiple mutations is demanding but necessary to examine functional redundancy in repair systems. Furthermore, live cell imaging methods[104], to assess the behaviour of single proteins in a bacterium during DNA repair, have not yet been adapted for use in microorganisms within the context of their hosts. Despite these limitations, it is clear that the BER pathways differ between the three bacterial pathogens discussed here. Compared to the minimal system in *H. pylori* (which lacks the MMR and NHEJ pathways), DNA repair in *M. tuberculosis* is characterized by remarkable redundancy, especially in the GO system. In addition, DNA repair enzymes in meningococcus display functional specialization for the repair of AP sites, but these enzymes function within a network of interacting repair pathways. An increase in our understanding of the mechanisms involved in BER in these and other human-adapted pathogens should provide unparalleled insights into the fundamental process of DNA repair and offer potential opportunities for the development of novel therapeutics.

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