Tuberculosis 91 (2011) 533–543

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

REVIEW

Base excision and nucleotide excision repair pathways in mycobacteria

Krishna Kurthkoti [a], Umesh Varshney [a,b,*]

[a] Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012, India
[b] Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore 560064, India

ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form
1 June 2011
Accepted 12 June 2011

Keywords:
Hypoxia
BER
NER
Ung
UdgB

SUMMARY

About a third of the human population is estimated to be infected with *Mycobacterium tuberculosis*. The bacterium displays an excellent adaptability to survive within the host macrophages. As the reactive environment of macrophages is capable of inducing DNA damage, the ability of the pathogen to safeguard its DNA against the damage is of paramount significance for its survival within the host. Analysis of the genome sequence has provided important insights into the DNA repair machinery of the pathogen, and the studies on DNA repair in mycobacteria have gained momentum in the past few years. The studies have revealed considerable differences in the mycobacterial DNA repair machinery when compared with those of the other bacteria. This review article focuses especially on the aspects of base excision, and nucleotide excision repair pathways in mycobacteria.

1. DNA repair in mycobacteria and the scope of the article

Cellular DNA is continually exposed to reactive radicals of intracellular or extracellular origin often leading to irreversible changes in the genetic blue print. If left unattended, such changes lead to mutations. Some of the well studied intracellular DNA damaging agents such as the reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) are generated during cellular metabolism. As the pathogenic bacteria have to establish and perpetuate within the host, the study of DNA repair in these bacteria forms an interesting model system. In fact, *Mycobacterium tuberculosis* which preferentially infects macrophages, the host's first line of defence in the immune system (which produce ROS and RNI), should possess a robust DNA repair machinery to ensure maintenance of the integrity of its genome to survive within the host and to establish a successful infection.[1–3] While as a part of its survival strategy the bacterium possesses an elaborate mechanism to detoxify the ROS and RNI,[4] any residual levels of these agents can be detrimental to the genomic integrity. A detailed knowledge of DNA repair mechanisms in pathogens may offer a basis to design therapies or prophylactics against them.

Availability of the genome sequences of some of the important human pathogens such as *Haemophilus influenzae*, *Helicobacter pylori*, *M. tuberculosis* has offered a new approach to advance our knowledge of DNA repair mechanisms in these organisms.[5–7] There is a growing body of evidence suggesting that pathogens often behave differently from *Escherichia coli* and *Bacillus subtilis* models in having a distinct representation of DNA repair enzymes.[8] For example, in *Campylobacter jejuni* that causes gastrointestinal infections, enzymes of direct repair pathway and base excision repair pathway are under-represented. In *Streptococcus pneumoniae* that colonizes upper respiratory tract, not only the direct and base excision repair proteins but also the members of recombinational repair are under-represented.

The genus mycobacterium consists of members that continue to have a great impact on the human society as important pathogens.[9] The prominent members include *M. tuberculosis*, the causative agent of tuberculosis and *Mycobacterium leprae* that causes leprosy. The genome of *M. tuberculosis* (~4.2 MB) has a high G + C content (~66%). Thus, considering a highly reactive environment of macrophage, there is a greater risk of cytosine deamination to uracil and guanine oxidation to 7,8-dihydro-8-oxoguanine (8-oxoG) or other oxidized intermediates. The *M. tuberculosis* genome sequence revealed,[7,10,11] that it contains many of the base excision and nucleotide excision repair genes found in *E. coli*. However, it lacks homologues of mismatch repair pathway genes.[7,10–12] Interestingly, the bacterium possesses ERCC3 (XPB) and Mpg enzymes, which were until then recognized exclusively in the mammalian cells. The bacterium also encodes homologues of the

* Corresponding author. Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012, India. Tel.: +91 80 2293 2686; fax: +91 80 2360 2697.
E-mail addresses: varshney@mcbl.iisc.ernet.in, uvarshney@gmail.com (U. Varshney).

1472-9792/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tube.2011.06.005

nonhomologous end joining pathway (NHEJ), which has been established as a major pathway for repairing double strand DNA breaks in eukaryotes. Proteins such as Ku 70, Ku 80, DNA ligase D that function in this pathway have been identified in *M. tuberculosis* and other bacterial members.[13,14] Expression of Ku (Rv0937c) increases in *M. tuberculosis* in the infected human samples.[15] Recently, two members of the Y family of polymerases (which perform translesion DNA synthesis) from *M. tuberculosis* DinP (Rv3056) and DinX (Rv1537), referred to as DinB2 and DinB1, respectively were also reported.[16]

DNA repair studies in mycobacteria make extensive use of *Mycobacterium smegmatis*, a non-pathogenic and a relatively fast growing member of the group, as a model. Similar to *M. tuberculosis*, *M. smegmatis* has a G + C rich genome (~67%), lacks mismatch repair genes, and the DNA repair proteins in *M. tuberculosis* and *M. smegmatis* share a high degree of similarity.[10,11] In addition, the availability of relatively more tractable genetic methods for *M. smegmatis* render it useful to obtain the first information report of the distinct features of DNA repair in mycobacteria.

As the members of mycobacteria do not encode homologues of the mismatch repair enzymes,[11] the base excision repair (BER) and the nucleotide excision repair (NER) pathways may play a major role in maintaining the integrity of DNA in these bacteria. Furthermore, because of the G + C richness of the genome, it is not surprising that there is more emphasis on enzymes that are involved in the excision of uracil and the oxidized guanine bases. In the recent past, several reviews[11,17] have addressed general aspects of DNA repair in mycobacteria. More recently, role of DNA repair in *M. tuberculosis* pathogenesis has also been reviewed.[18] In this article, we restrict ourselves to the salient features of BER and NER pathways in mycobacteria. A list of various proteins involved in these pathways is provided in Table 1.

2. BER pathway

The BER pathway involves excision of modified bases in DNA by a class of enzymes called DNA glycosylases which are highly evolved to specifically recognize the modified/damaged base(s) in the context of DNA backbone to initiate BER. A number of DNA glycosylases are monofunctional enzymes which hydrolyze the N-glycosidic bond between the base and the sugar and result in the formation of abasic (AP) sites.[19] However, the bifunctional DNA glycosylases, in addition to hydrolyzing the N-glycosidic bond, continue to cleave the phosphodiester backbone at the AP sites by their lyase activity. Presence of unprocessed AP sites can be more detrimental than the modified bases as they impede the essential cellular processes such as replication and transcription. The accumulation of AP sites in DNA is both mutagenic and cytotoxic.[20–22] A class of enzymes called AP endonucleases (APE) act on AP sites to cleave the phosphodiester bond resulting in a nick in the DNA. The nicks so generated require further processing before the ends of the DNA can be used by the downstream processing enzymes. A major pathway for processing of the AP sites in *E. coli* employs exonuclease III or endonuclease IV to hydrolyse the phosphodiester bond 5′ to the abasic deoxyribose sugar to generate a 3′ hydroxyl, and a 5′ deoxyribose ends. A deoxyribosephosphodiesterase (dRpase) activity (*e. g.* RecJ) is then utilized to cleave the deoxyribose and generate a 5′ phosphate end.[23,24] The 5′ phosphate end can also result from β-elimination of the deoxyribose, a reaction promoted by Fpg.[25] The single nucleotide gap surrounded by 3′ hydroxyl and 5′ phosphate ends is filled in by DNA polymerase I and sealed by DNA ligase to restore the original sequence.[23,24] The lyase activity of the bifunctional DNA glycosylases or the AP lyases (*e. g.* endonuclease III) may also process the AP sites by cleaving 3′ to the abasic sugar to generate a 5′ phosphate end. Although such a reaction bypasses the requirement of dRpase, it requires further processing of the 3′ end (*e. g.* by exonuclease III or endouclease IV) to convert the 3′ unsaturated aldehyde (indicated by 3′ PA in Figure 1) to a 3′ hydroxyl end to serve as primer for the DNA polymerase.[26,27] In an alternate pathway, even though the 5′-3′ exonuclease activity of DNA polymerase I is unable to remove the 5′ deoxyribose, alternate DNA polymerase(s) (*e. g.* as known in eukaryotes) may carry out the fill in reaction (from the 3′ hydroxyl end) by replacement synthesis. The 5′ flank containing the deoxyribose residue may then be removed as a part of a DNA oligomer by a structure specific endonuclease activity (*e. g.* FEN-1) followed by sealing of the nick (containing 3′ OH and 5′ PO_4) by DNA ligase.[24] Thus, while the predominant BER pathway results in single nucleotide repair patch (Figure 1, *left panel*), the alternate pathway would lead to a multiple nucleotide repair patch (Figure 1, *right panel*). A recent study shows a repair size of more than one nucleotide in mycobacteria.[28] However, it would be interesting to investigate the mechanism of this longer size patch repair in mycobacteria.

2.1. DNA glycosylases involved in uracil excision in mycobacteria

Uracil DNA glycosylases (UDGs) excise uracil formed either due to deamination of cytosine or misincorporation of dUTP (as dUMP against A) during replication.[29] Inability to repair uracil arising from cytosine deamination leads to G:C to A:T transitions. Although, direct incorporation of dUMP is not mutagenic in itself, it may impact protein binding to DNA. The incorporation of dUMP in genome is kept to a minimum by dUTPase (encoded by *dut*) which hydrolyses dUTP to minimize the cellular pools of dUTP and thereby reducing the chances of its misincorporation by DNA polymerases.[30,31] Loss of dUTPase leads to increased incorporation of uracil in DNA and may be lethal or result in elevated mutation rates.[32,33] *M. tuberculosis* and the other members of mycobacteria have been shown to contain *dut*.[11] *M. tuberculosis* Dut (Rv2697c) has been shown to be a bifunctional protein possessing dCTPase and dUTPase activities,[34] and the *dut* gene has been identified as an essential gene in a transposon mutagenesis screen.[35]

A number of UDG activities have been identified in various organisms. Of these, the Ung proteins (family 1 UDGs) are the most conserved, highly efficient and by far the best characterized UDGs. The family 1 UDGs excise uracil from both the single-stranded and double-stranded DNAs, and possess two highly conserved amino acid sequence motifs, A and B (GQDPY and HPSPLS, respectively).[36–39] The Ung proteins are potently inhibited by a *B. subtilis* phage protein, Ugi.[40] The family 2 UDGs (also known as dsUDGs, MUG or DUG) which excise uracil or thymine from G:U/T mismatches in dsDNA, possess GINPG and MPSSSAR as motifs A and B, respectively.[41] The single stranded DNA selective monofunctional UDGs (SMUG, family 3) possess GMNPG and HPSPRNP as motifs A and B, respectively.[42] SMUG also acts on dsDNA but requires AP endonuclease for the product release. Two more UDGs, UdgA (family 4) containing GEAPG and HPAAVLR, and UdgB (family 5) having GLAPA and HPSPLNV as motifs A and B, respectively have been characterized.[43–46].

Mycobacteria possess family 1 and family 5 UDGs (Table 1). Family 1 UDG from *M. smegmatis*,[47] though similar to *Eco*Ung in its activities, possesses certain novel characteristics. For example, compared to *Eco*Ung, *Msm*Ung is more efficient in excising uracils from the loop substrates. This property may be of particular significance in mycobacteria because of high G + C contents of its genome, which may lead to formation of more stable looped DNA structures. The three dimensional structure of *Mtu*Ung with Ugi has been determined.[48] The structure of the central core of *Mtu*Ung is similar to the Ung from other sources. However, its N- and C-terminal tails show variability and its DNA-binding region is rich in arginine residues. Whether these structural features are

Table 1
List of proteins involved in BER and NER in *M. tuberculosis*, *M. smegmatis*, and *M. leprae*. Gene annotations corresponding to these proteins adapted from Davis and Forse[11] are as shown in the second column from left (Rv, *M. tuberculosis*; MSMEG, *M. smegmatis*; and ML, *M. leprae*). In this column gene annotations followed by psg, indicates psuedogene; and ML– indicated lack of the corresponding gene homolog in *M. leprae*.

Protein class/name	Gene annotations in *M. tuberculosis* (Rv), *M. smegmatis* (MSMEG) and *M. leprae* (ML)	Comments	References
1. Base excision repair			
(a) DNA glycosylases			
Ung	Rv2976c, MSMEG_2399, ML1675c	Class 1 Uracil DNA glycosylase. Biochemical, structural and function analysis, and *in vitro* repair in mycobacterial cell-extracts have been reported. Reconstitution with purified proteins has not been done. *M. smegmatis* strain deficient in Ung shows compromised viability under conditions of various DNA damages. Characterization of Ung deficient strains of *M. tuberculosis* has not been reported.	28, 47, 50, 56, 59, 60
UdgB	Rv1259, MSMEG_5031, ML1105	Class 5 Uracil DNA glycosylase. Biochemical characterization of *M. tuberculosis* and *M. smegmatis* UdgB has been carried out. *M. smegmatis* deficient in UdgB alone is not significantly compromised in its phenotype. However, together with Ung deficiency it leads to high susceptibility of the strain to various DNA damaging agents. UdgB complements for Ung deficiency in *E. coli* as well as *in vitro* in mycobacterial cell-free extracts for uracil excision repair. However, the primary *in vivo* substrate of UdgB may be deaminated adenosine. *M. tuberculosis* strains deficient in Ung and/or UdgB have not been reported.	
Fpg (MutM)	Rv2924c, MSMEG_2419, ML1658c	Formamidopyrimidine DNA glycosylases, a bifunctional (glycosylase/lyase) enzyme. Biochemical characterization of mycobacterial Fpg proteins has been carried out. Repair pathway has not yet been reconstituted from the purified proteins. Fpg deficient *M. smegmatis* shows an increase in mutation frequency and altered mutation spectrum. The genes for Fpg2 may be pseudogenes. *M. tuberculosis* strains deficient in Fpg have not been characterized.	63–66
Fpg2	Rv0944, MSMEG_5545, ML0148 psg		
Nei1	Rv2464c, MSMEG_4683, ML1483c	Endonuclease VIII, a bifunctional (glycosylase/lyase) enzyme acting on oxidized pyrimidines on both double stranded, and single stranded DNA. Identified uracil DNA glycosylase activity of this protein may need further validation by purification of the protein from an *ung*- strain/assays in the presence of Ugi.	
Nei2	Rv3297, MSMEG_1756, ML–	Endonuclease VIII 2. The recombinant protein (Rv3297) did not show biochemical activity but its expression in *E. coli* reveals functional activity on oxidation products of cytosine and guanosine. Further, characterization of the protein and its interacting partners may reveal interesting aspects of the Nei2.	
Nth	Rv3674c, MSMEG_6187, ML2301c	Endonucease III, excises 5, 6-dihrouracil, 5-hydroxyuracil, 5-hydroxycytosine, methylhydantoin, and thymine glycol.	
MutY	Rv3589, MSMEG_6083, ML1920	Adenine DNA glycosylase. Biochemical characterization of *M. smegmatis* and *M. tuberculosis* MutY has been reported. Deficiency of MutY alone in *M. smegmatis* does not result in increased mutation frequency. However, there is an increase in the expected C to A mutations. Characterization of *M. tuberculosis* deficient in MutY/Fpg in singles or combination is pending.	72
TagA	Rv1210, MSMEG_5082, ML1066	Identified based on sequence homologies with their counterparts in other organisms. In mycobacteria, AlkA is part of AdaA-AlkA composite protein. Characterization of this protein suggests that it lacks alkylbase DNA glycosylase activity, although it possesses methyltransferase activity. Functional and biochemical characterizations of TagA and Mpg have not been reported. It would be reasonable to propose that these two enzymes may play a major role as glycosylases that remove alkylated bases.	17, 84
AlkA	Rv1317c,MSMEG_4925, ML1152c psg		
Mpg	Rv1688, MSMEG_3759, ML1351		
(b) AP-endonucleases			
Nfo	Rv0670, MSMEG_1383, ML1889c	Predicted homologs of endonuclease IV (Nfo) and exonuclease III (Xth). Biochemical and functional characterization of these proteins has not been reported from any mycobacterial species.	10, 11
Xth	Rv0427c, MSMEG_0829, ML1931 psg		
(c) NTPases/dNTPases			
Dut	Rv2697c, MSMEG_2765, ML1028	*M. tuberculosis* Dut has been purified and its three dimensional structure determined. The enzyme shows both dUTPase and dCTPase activitites.	11, 34, 35
MutT1	Rv2985, MSMEG_2390, ML1682	Identified based on *E. coli* MutT which hydrolyses 8-oxo(dGTP)/8-oxo(GTP). Mutants of *M. tuberculosis* and *M. smegmatis* deficient in either of the MutT proteins have been studied. MutT1 and MutT2 possess 8-oxo-dGTPase activity. MutT2 also possesses strong activities of hydrolyzing dGTP and dTTP. MutT4 possesses dATPase activity. MutT2 has also been shown to possess dCTPase activity. Detailed biochemical characterization of the MutT proteins is desirable to understand their physiological role. It is unclear if the multiple dNTPases/NTPases facilitate to down regulate metabolism during dormancy/persistence.	76–78
MutT2	Rv1160, MSMEG_5148, ML1503c psg		
MutT3	Rv0413, MSMEG_0790, ML0301c psg		
MutT4	Rv3908, MSMEG_6927, ML2698		
RdgB	Rv1341, MSMEG_4899, ML1175	Identified as NTPase responsible for removal of hypoxanthine or xanthine triphosphates. Biochemical and functional characterization of this protein from mycobacteria have not been reported.	11

(*continued on next page*)

Table 1 (*continued*)

Protein class/name	Gene annotations in *M. tuberculosis* (Rv), *M. smegmatis* (MSMEG) and *M. leprae* (ML)	Comments	References
MazG	Rv1021, MSMEG_5422, ML0253 psg	*M. tuberculosis* and *M. smegmatis* proteins are nucleoside triphosphate pyrophosphohydrolases which hydrolyze all canonical dNTPs, dUTP and 8-oxo-dGTP. *M. smegmatis* deficient in MazG are sensitive to oxidative stress.	79
2. Nucleotide excision repair			
(a) Exinuceases/related proteins			
UvrA	Rv1638, MSMEG_3808, ML1392	Proteins identified based on homology with their counterparts from other organisms. *M. smegmatis* has two more proteins UvrA2 (MSMEG_6808) and UvrA like protein (MSMEG_0211) whose functions are not known. *M. tuberculosis* and *M. smegmatis* deficient in UvrB have been shown to be susceptible to UV and other DNA damaging agents. *M. tuberculosis* strain deficient in UvrB has compromised virulence in mice. Biochemical characterization of *M. tuberculosis* Mfd shows that unlike the *E. coli* counterpart the *M. tuberculosis* protein is found distributed between monomeric and hexameric forms. The extreme C-terminal region of the Mfd was shown to be responsible for its oligomerization. Such a distinct property of Mfd suggests a novel mechanism of transcription coupled repair in mycobacteria.	51, 97, 106
UvrB	Rv1633, MSMEG_3816, ML1387		
UvrC	Rv1420, MSMEG_3078, ML0562		
Mfd	Rv1020, MSMEG_5423, ML0252		
(b) Helicases			
UvrD1	Rv0949, MSMEG_5534, ML0153	UvrD1 was identified on the basis of its homology *E. coli* UvrD. Has a preferential duplex-unwinding activity with 3′-to-5′ polarity from nicked DNA duplexes and stalled replication forks. UvrD2, an atypical protein, is also a 3′ to 5′ helicase. UvrD2 is essential in *M. smegmatis*. Both the proteins may have overlapping functions but UvrD2 may be crucial for essential functions. Interestingly, both the proteins show high conservation in the clinical samples.	108, 118–120
UvrD2	Rv3198c, MSMEG_1952, ML0637		
XPB (ercc3)	Rv0861c, MSMEG_5706, ML2157	XPB from *M. tuberculosis* has been biochemically characterized. It shows an ATP dependent 3′-5′ helicase activity. The *in vivo* function of this protein needs to be elucidated.	109, 110
3. DNA polymerases (repair)			
PolA	Rv1629, MSMEG_3839, ML1381c	PolA is the major DNA polymerase that fills the gaps resulting from AP endonuclease activity. Disruption of *polA* in *M. smegmatis* results in hypersensitivity to DNA damage induced by UV irradiation and by hydrogen peroxide challenge. Mycobacteria lack DNA polymerase II, but possess DnaE2 as the error prone DNA polymerase. Deletion of DnaE2 in *M. tuberculosis* results in hypersensitivity to DNA damages and eliminates damage-induced mutagenesis *in vitro*. It is a major mediator of induced mutagenesis in mice, and known to play a role in the emergence of drug resistance. Mycobacteria also possess DinP and DinX predicted equivalents of the error prone Y-family DNA polymerases V and IV, repectively to carry out translesion synthesis across the damaged sites. Genetic analyses in *M. tuberculosis* reveal that the DinP and DinX behave differently from their counterparts from other organisms.	16, 17, 81, 121, 122
DinP (DinB2)	Rv3056, MSMEG_2294, ML1739 psg		
DinX (DinB1)	Rv1537, MSMEG_3172, ML1197 psg		
DnaE2	Rv3370c, MSMEG_1633, ML0416 psg		
4. DNA Ligases			
LigA	Rv3014c, MSMEG_2362, ML1705	All four DNA ligases have been characterized. LigA is NAD(+)-dependent whereas the other three (LigB, LigC, and LigD) are ATP dependent. LigA and LigB show a strong nick sealing activity as opposed to the weak nick sealing activity of LigC and LigD. The three ATP-dependent ligases are not essential for the growth of *M. tuberculosis*. However, the LigD deficient cells are defective in nonhomologous DNA end joining. Specific contributions of each ligase in base excision and nucleotide excision repair pathways are not known.	14, 123
LigB	Rv3062, MSMEG_2277, ML1747 psg		
LigC	Rv3731, MSMEG_6302, ML–		
LigD	Rv0938, MSMEG_5570, ML2090c psg		

responsible for the distinct activities of mycobacterial Ung and its interaction with Ugi[48] has not been analyzed. Interestingly, the sequence micro-heterogeneity in the active site pocket of *Mtu*Ung proved useful to understand the mechanistic aspects of the substrate binding to the Ung proteins.[49] *M. smegmatis* deficient in Ung revealed ~9 fold increase in the mutation rates over the wild-type parent.[50,51] These studies also demonstrated that the mutant was sensitive to acidified nitrite (a source of RNI) and the macrophage environment that produces RNI and ROS. The importance of Ung was further supported by a generalized screen in *M. tuberculosis*[52] wherein a pathogenic strain deficient in Ung showed a reduced fitness in the mouse infection model. It has been reported that single stranded DNA-binding protein (SSB) from mycobacteria influences Ung activity, by interacting through its (SSB) C-terminal domain.[53,54]

The Rv1259 from *M. tuberculosis* was predicted[55] to encode the family 5 UDG (now named *Mtu*UdgB). Subsequently, the *Mtu*UdgB has been shown to contain an iron sulphur cluster and, unlike the family 1 Ung, it exhibits broader substrate specificity.[56] In addition to uracil, it excises ethenocytosine and hypoxanthine from dsDNA. Unlike the family 1 Ung, *Mtu*UdgB displays thermotolerance and insensitivity to Ugi. Biochemical analysis revealed that the purified enzyme shows poor turnover. However, upon supplementation with cellular extracts, the enzyme exhibits increased turnover indicating the role of additional factors in the repair pathway. Thus, *Mtu*UdgB may serve as a good model to investigate the mechanism of possible occurrence of a coupled reaction between Ung and the AP-endonuclease.[57,58] *M. smegmatis* contains an orthologue of *Mtu*UdgB encoded by MSMEG_5031.[59] Using *M. smegmatis* strains deficient in Ung and UdgB, it was reported that the *udgB* mutant is

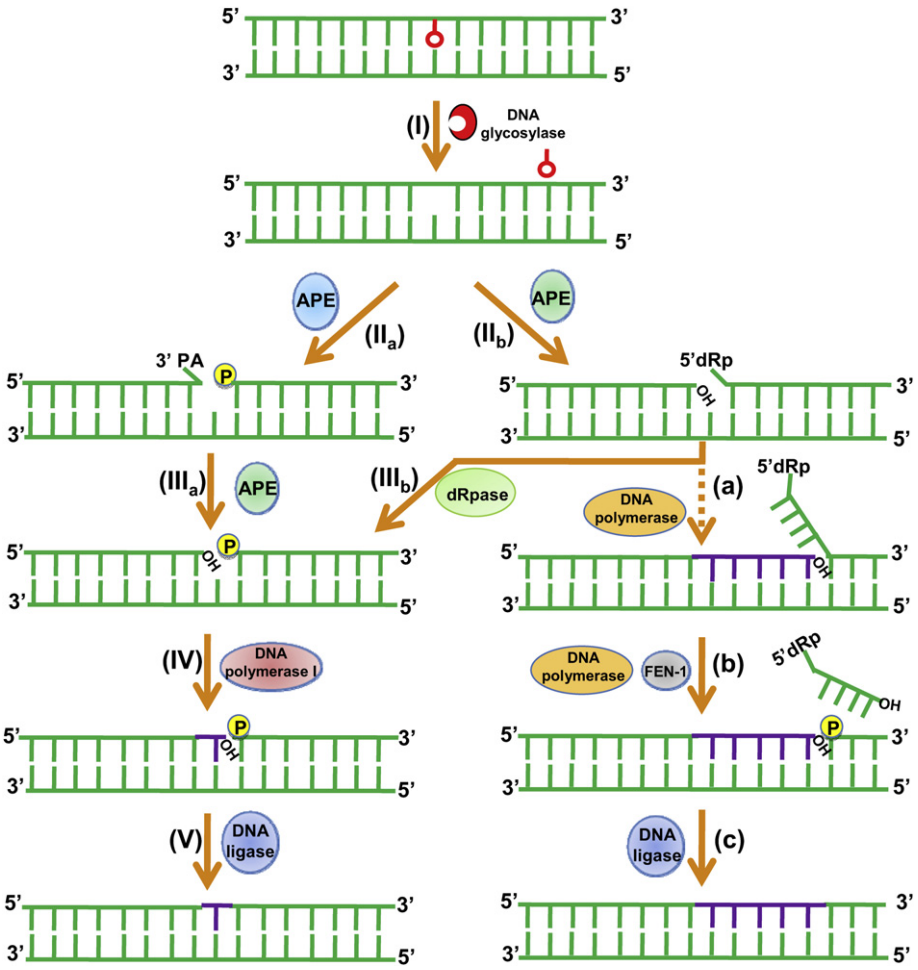

Figure 1. Scheme of base excision repair pathway. A double stranded DNA containing a damaged/modified base (indicated by a hanging circle in red) is shown on the top. The damage is identified by a DNA glycosylase (step I) which hydrolyzes the N-glycosidic bond between the base and the sugar and results in the formation of an abasic (AP) site. Action of AP endonucleases (APE) and deoxyribosephosphodiesterase (dRpase) through steps II_a and III_a or II_b and III_b results in the formation of a single nucleotide gap with 3′ OH and 5′ phosphate ends suitable for filling in by DNA polymerase I (step IV) and ligation (step V) to give rise to the repaired DNA. In an alternate pathway, product of step II_b containing a 3′ OH end may be utilized by alternate DNA polymerase (s) to carry out fill in reaction by replacement synthesis (step a), and remove the 5′ flank containing the deoxyribose residue as a part of the DNA oligomer by a structure specific endonuclease activity, replacing the strand containing the 5′ dRp end (step b) and generating a nick in DNA which is sealed by the ligase (step c). While the predominant pathway (*left panel*) results in single nucleotide repair patch, the alternate pathway may lead to a multinucleotide repair patch (*right panel*).See text for further details.

not as sensitive to DNA damaging agents as the *ung* mutant is.[59,60] However, a double deficiency of Ung and UdgB results in synthetic effects resulting in increased mutation rates, and hypersensitivity to acidified sodium nitrite and 5-fluorouracil. In addition, the mutation spectrum analysis of the UdgB mutants of *M. smegmatis* in these studies confirmed the *in vivo* relevance of uracil and hypoxanthine excision by UdgB. These observations may have significant implications to generation of attenuated strains of *M. tuberculosis*. UdgB orthologs have been found in many of the mycobacterial members by sequence analysis.[11]

2.2. DNA glycosylases involved in oxidative damage repair

Guanine is highly sensitive to oxidative agents and results in the formation of 8-oxoG. If 8-oxoG is left unrepaired, G:C to T:A transversions arise because of incorporation of A against 8-oxoG. Organisms maintain an elaborate oxidative damage repair system called the GO repair pathway.[61] The formamidopyrmidine DNA glycosylase (Fpg, also called MutM) excises oxidized purines, and displays AP lyase activitiy.[62] Mycobacteria contain all proteins (MutM, MutY and MutT) specific to the GO repair pathway.[7,10,11] Another class of enzymes that excises oxidized bases is endonuclease VIII (Nei). While Fpg acts primarily on oxidized purines, Nei enzymes act primarily on oxidized pyrimidines. Along with the classical Fpg (MutM, Rv2924c) and Nei (Rv2464c), *M. tuberculosis* genome contains new homologues of these enzymes; namely, Fpg2 (Rv0944) and Nei2 (Rv3297). A study of Fpg (MSMEG_2419) deficient *M. smegmatis*[63] has shown that Fpg plays an important role in protecting the bacteria from oxidative DNA damage, and the *M. tuberculosis* Fpg (Rv2924c) could complement this defect. It was shown that the cell free extracts of wild-type *M. smegmatis* could excise 8-oxoG when base paired against C, G or T. Analysis of the mutation spectrum of Fpg deficient strain showed distinct mutations in the rifampicin resistance determining region (RRDR) of *rpoB*. Interestingly, loss of Fpg displayed an increase in A to G mutation but not the expected C to A mutations. The C to G mutations increased when the strain was subjected to sub-lethal oxidative stress. Biochemical analysis using cell free extracts revealed that deviations in the mutation spectrum could be due to a preferential incorporation of G against 8-oxoG by DNA polymerase(s). The *Mtu*Fpg (Rv2924c, also termed as *Mtb*-Fpg1) excises 8-oxoG from double stranded DNA substrates, when base paired

against C, G or T but not A.[64,65] The purified protein demonstrated the formamidopyrimidine (faPy) glycosylase and the AP lyase activities. The other member, *Mtu*Fpg2 may be a psuedogene as it lacks the highly conserved N-terminal proline residue that forms a part of the catalytic centre.[65]

Biochemical characterization of *Mtu*Nei1(Rv2464c)[65] shows that it excises thymine glycol and 5,6-dihydrouracil (DHU) residues and possesses AP lyase activity. It was also shown that *Mtu*Nei1 could complement for Fpg or MutY deficiency in *E. coli* and significantly reduce the spontaneous mutation rates. Sidorenko et al.[66] reported similar enzymatic properties, for the Nei that they termed as Nei2. However, considering that the oligonucleotides used for amplification of the gene corresponded to *Mtu*Nei1 (Rv2464c)[65] the protein characterized by Sidorenko et al., should be considered *Mtu*Nei1. The other protein, *Mtu*Nei2 (Rv3297) was shown to rescue the mutator phenotype of *E. coli* deficient in Fpg, MutY or Nei.[65] *Mtu*Nth (Rv3674c), which can process oxidized pyrimidine residues has also been biochemically characterized[65] and shown to act on 5,6-dihydrouracil (DHU), 5-hydroxyuracil and 5-hydroxycytosine residues.

The adenine DNA glycosylase (MutY) is another important player in the GO repair pathway. This iron sulphur cluster containing protein is unique among DNA repair enzymes as it removes the normal base, adenine, when paired against 8-oxoG, G or C.[67–69] Presence of an A against 8-oxoG could be a consequence of its misincorporation by DNA polymerase(s). The adenine DNA glycosylase activity of MutY increases the chances of incorporation of the correct base (C) against 8-oxoG, as well as the probability of further repair of the lesion (8-oxoG) by Fpg. Thus, the action of MutY prior to a new round of DNA replication provides an efficient mechanism to decrease fixation of A or G in place of C in the genome, and prevents C to A and to a lesser extent C to G mutations. The C-terminal domain of MutY is involved in recognition of the DNA lesion and it shares structural similarity with MutT.[70,71] The N-terminus containing the 4Fe-4S cluster is involved in the catalysis of adenine excision. MutY has also been reported to possess a weak AP endonuclease activity.[68] The genome sequences of all the mycobacterial members predict the presence of MutY.[11] The biochemical characterization of *Mtu*MutY shows it to be similar to the *E. coli* protein.[72] However, the loss of MutY in *M. smegmatis* did not lead to a significant effect on the mutation rate or its sensitivity to oxidative stress. Biochemical characterization of mycobacterial MutY (*M. smegmatis* and *M. tuberculosis*) revealed an expected excision of A opposite 8-oxoG in DNA. Additionally, a detectable excision of G and T opposite 8-oxoG was noted. The MutY formed complexes with DNA containing 8-oxoG × A, 8-oxoG × G or 8-oxoG × T but not 8-oxoG × C pairs. The MutY deficient strain showed distinct mutations in the RRDR of *rpoB*. Besides the expected C to A (or G to T) mutation, an increase in A to C mutations was observed. Primer extension reactions performed with the cell free extracts of the wild-type bacteria showed the ability of the DNA polymerase(s) to misincorporate 8-oxoG against A, C and T. Incorporation of 8-oxoG against normal nucleotides is carried out by the Y family DNA polymerases[73] and *M. smegmatis* is predicted to encode at least four such proteins.[11] The biochemical investigation of these polymerases is pending. Nonetheless, findings from cell extract experiments along with the earlier observations of preferential incorporation of G by mycobacterial polymerases have provided a better understanding of the mutation spectrum. It would be worthwhile to study the phenotypes of mycobacterial strains deficient in both Fpg and MutY. Interestingly, mutant strains of *M. tuberculosis* lacking either *fpg* or *nei* showed attenuation in a primate model of infection.[74] These results further highlight the importance of oxidative DNA damage repair pathway and provide a rationale as to why these bacteria encodes multiple enzymes for a single pathway.

MutT is a member of Nudix (**nu**cleotide **di**phosphate linked to **X**) family of proteins. This enzyme plays an important role in preventing misincorporaion of the oxidized dGTP (or dNTPs) by hydrolyzing them into nucleoside monophosphates and pyrophosphates. MutT from *E. coli* has been characterized[75] and shown to have high specificity of cleaving oxidized dGTP into dGMP and PPi. The genome of *M. tuberculosis* encodes four MutT like proteins.[7,11,76] In *M. tuberculosis* and *M. smegmatis,* knockouts of different MutTs lead to increased mutation rates.[76] Presence of missense mutations in *mutT2* and *mutT4* in the *M. tuberculosis* W-Beijing isolates has been reported. However in these strains, mutations in *mutT* could not be linked to the high drug resistance.[77] Recently, it has been shown that the *Mtu*MutT2 (closest to *E. coli* MutT) has a strong dCTPase activity.[78] In fact, in *M. smegmatis* the orthologue of *M. tuberculosis* MutT2 is annotated as a dCTPase. However, one or more of the remaining MutTs could actually possess 8-oxoGTPase activities. Nevertheless, the occurrence of A to G mutations in the wild-type and an increase in A to G and A to C mutations in MutY deficient strains indicates that the steady state levels of 8-oxo-dGTP may be high in mycobacteria.[72] In addition, mycobacteria also possess RgdB and MazG (Table 1). Of these, MazG hydrolyzes all canonical dNTPs, dUTP and 8-oxo-dGTP. *M. smegmatis* deficient in MazG are sensitive to oxidative stress.[79] It would be interesting to see if the multiple NTPases/dNTPases in *M. tuberculosis* play a physiologically relevant role during dormancy by depleting nucleotide pool.

2.3. Glycosylases involved in repair of alkylated DNA bases

Agents such as methylmethane sulfonate or dimethylnitrosoamine, alkylate DNA bases. TagA acts on alkylated bases such as 3-methyladenine.[80] AlkA is another glycosylase with broad substrate specificity acting on 3-methylpurines and 7-methylpurine in DNA. Each of the mycobacterial members is predicted to contain homologue of *tagA*. AlkA homologue of *M. tuberculosis* (Rv1317c) has been shown to be significantly upregulated upon oxidative stress[81] and to a lesser extent following infection to macrophages.[82] In *M. tuberculosis,* AlkA is part of AdaA-AlkA composite protein. Recently, characterization of this protein[84] suggested that it lacks alkylbase DNA glycosylase activity, although it possesses methyltransferase activity. Also, a mutant of *M. tuberculosis* lacking AlkA was not compromised for its growth in mouse organs.[83] Another gene that shows higher similarity to the eukaryotic 3-methyladenine glycosylases, *mpg* (Rv1688), is present in many mycobacterial members[11] (Table 1). Biochemical or mutational analysis for this gene is currently unavailable but the expression of *mpg* has been reported to be elevated in clinical samples of *M. tuberculosis*.[15]

2.4. AP endonucleases in mycobacteria

Action of DNA glycosylases leaves behind an abasic or AP (apurinic/apyrimidinic) site. These AP sites inhibit the movement of replicative polymerase and stall the replication fork.[29,85] In *E. coli* the endonuclease IV (Nfo) and exonuclease III (Xth) cleave the phosphodiester bond 5′ to the abasic deoxyribose sugar to generate a 3′ hydroxyl, and a 5′ deoxyribose ends at the site of damage. Alternatively, action of endonuclease III cleaves 3′ to the AP site generating a 5′ phosphate end. The Xth is the major AP endonuclease in *E. coli*.[86] Loss of *xth/nfo* in *Salmonella typhimurium*, leads to its attenuation in mice model of infection indicating the importance of abasic damage repair for bacterial survival.[87] In mycobacterial members the *xth* and *nfo* have been identified. The XthA is not seen in *M. leprae* where it has been converted into a psuedogene.[11] However, the *M. smegmatis* genome sequence predicts an additional homologue of XthA.[11] Importance of Xth and Nfo are highlighted by their requirement for bacterial survival in the initial

stages of infection in mouse models as revealed by the high density transposon mutagenesis.[52]

3. The nucleotide excision repair in (NER)

The NER pathway (Figure 2) was discovered in the context of the repair of UV generated DNA damage.[88,89] Because of this reason the genes that participate in this pathway have been named *uvr* genes. Loss of the *uvr* genes confers sensitivity to UV generated DNA damages. During NER an oligonucleotide of 12–13 bases in the case of prokaryotes[90] and 22-24 nucleotides[91] in eukaryotes is often excised. The UvrA, as a dimer ($UvrA_2$) can bind non-specifically to the damaged DNA duplex with a relatively low affinity.[92,93] UvrB forms a complex with ($UvrA_2$) in an ATP dependent process and ($UvrA_2$) (UvrB) ternary complex recognizes the damage in the DNA. Recently, it has been proposed[94] that a complex of two molecules each of UvrA and UvrB scans the DNA for damage. Once the damage has been recognized one of the UvrB is tightly bound to the DNA and the other remains bound to the complex. UvrC, the endonuclease of the pathway, is then recruited to nick the DNA strand at 4th or the 5th nucleotide 3′ to the damaged site followed by a nick at the 7th or 8th nucleotide 5′ to the damaged site. UvrD, then removes the damaged piece of DNA by disassembling the UvrB, UvrC and the damaged DNA. The DNA polymerase I (PolI) fills the gap following which the action of DNA ligase establishes the phosphodiester linkage.

Homologues of the *uvrA*, *uvrB*, and *uvrC* are present in all the mycobacterial genomes sequenced, implying that NER is an important pathway. *M. smegmatis* genome is predicted to encode a second copy of UvrA, MSMEG_6806 (Table 1). Biochemical analysis for this protein is currently not available. It has been shown that upon exposure to hydrogen peroxide the expression of *uvrA* and *uvrB* increases in *M. tuberculosis*.[81] Similarly, these genes are upregulated when phagocytosed by human macrophages.[95] The *uvrC* that encodes the endonuclease was shown to be upregulated in clinical lung samples.[15] The *uvrB* (Rv1633) mutant of *M. tuberculosis* exhibited severe sensitivity to acidified sodium nitrite and UV, and reduced ability to grow in mouse model of

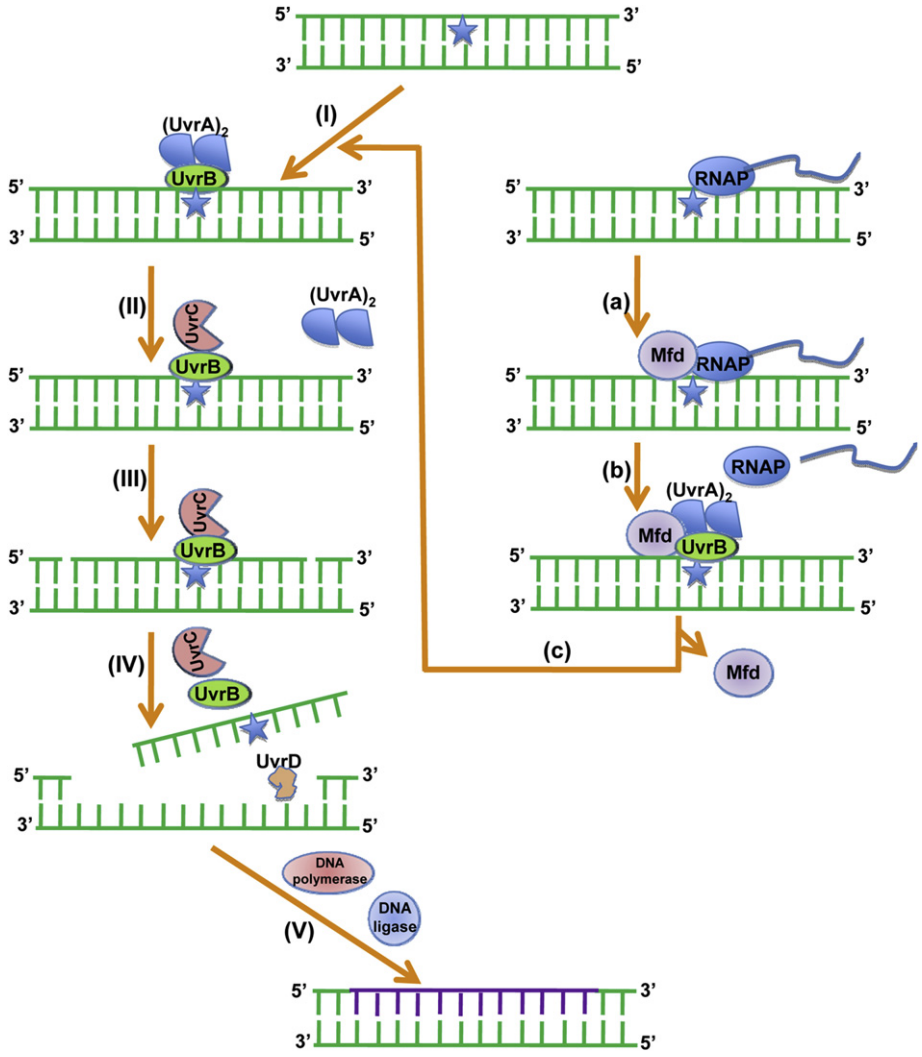

Figure 2. Scheme of nucleotide excision repair in eubacteria. A bulky damage in DNA (indicated by star) is recognized by the scanning ternary complex of $(UvrA)_2UvrB$ (step I). Identification of damage by UvrB leads to dissociation of $(UvrA)_2$ and recruitment of UvrC (Step II). The exinuclease activity of UvrC cleaves DNA both at the 5′ and the 3′ sides of the damage (Step III). Action of UvrD helicase leads to removal of UvrB and UvrC proteins along with the cleaved fragment containing the damage (~ 12-13 base oligonucleotide) (Step IV). DNA synthesis by DNA polymerase followed by ligation (step V) restores the integrity of DNA. Transcription coupled repair (shown in the right panel) is initiated when a transcribing RNA polymerase encounters a damage in the DNA which stalls the movement of RNA polymerase (RNAP). The stalled complex is recognized by Mfd (step a) leading to dissociation of RNAP and the truncated mRNA, and recruitment of $(UvrA)_2UvrB$ complex (Step b). Mfd is released (step c) and the $(UvrA)_2UvrB$ bound damage is repaired through steps II –V (shown in the left panel). See text for further details.

infection[96,97] and also in non human primate model of infection.[74] The *uvrC* has also been shown to be essential for *in vitro* growth in different systems.[98,35] Similarly, the loss of *uvrB* in *M. smegmatis* resulted in increased sensitivity to DNA damaging agents such as UV, RNI, ROS and hypoxia.[51] The comparative analysis of the significance of BER (Ung and Fpg) and NER pathways showed that among these pathways, NER played a more crucial role in preventing the detrimental effects of various DNA damaging agents.

In *E. coli*, a homologue of UvrC has been identified and termed Cho (C homologue). It shares sequence similarity with N-terminal portion of UvrC. Like UvrC, Cho catalyzes incision 3′ to the damage but four bases away from it.[99] A protein with homology to the N-terminal region of UvrC has also been identified in *M. tuberculosis* (Rv2191). Sequence analysis shows that the protein contains a domain with similarity to the proofreading domain of DNA polymerase III. Expression of this gene has been noted to be upregulated upon DNA damage *in vitro*[100] and in activated macrophages.[82] Further, the expression of Rv2191 has been reported to increase during dormancy and reactivation process in a rabbit model of infection[101] suggesting its importance during the course of infection. Interestingly, loss of *uvrB* and *uvrD1* in *M. smegmatis* led to increased frequency of gene integration demonstrating an important role for NER system in controlling recombination.[102]

3.1. Transcription repair coupling factor (Mfd)

The damaged DNA poses a problem to the transcription process by stalling the RNA polymerase. Studies in *E. coli* reported increased NER with a strong strand bias towards the transcribed strand.[103] The search for a factor that is involved in repair involving transcription complex led to the finding of Mfd (**m**utation **f**requency **d**ecline) in *E. coli* extracts.[104] Experiments with purified Mfd, UvrABC and RNA polymerase suggested that Mfd is able to recognize and interact with damaged DNA containing a stalled transcription unit. This leads to displacement of stalled RNA polymerase and the transcript. Mfd bound to DNA recruits ($UvrA_2$) (UvrB) and thus results in strand specific DNA repair. A homologue of Mfd is present in most mycobacterial members except the *Mycobacterium avium* subsp. *avium*.[11] Expression of *mfd* (Rv1020) increases upon infection to mouse or human macrophages.[15,105] Recent biochemical characterization of *M. tuberculosis* Mfd shows that unlike the *E. coli* counterpart the *M. tuberculosis* protein is found distributed between monomeric and hexameric forms. Also, the extreme C-terminal region of the Mfd was shown to be responsible for its oligomerization. Such a distinct property of Mfd suggests a novel mechanism of transcription coupled repair.[106] Deficiency of Mfd in *C. jejuni* resulted in increased sensitivity of the mutants to fluoroquinolone antibiotics.[107] It could be interesting to investigate the role of mycobacterial Mfd in conferring resistance to fluoroquinilones. The eukaryotic homologue of ERCC3 family of helicase that is involved in NER has also been observed in mycobacterial members.[10,11]

3.2. DNA helicase II or UvrD

The helicase function of UvrD disassembles UvrC (post incision) allowing the DNA polymerase to perform repair synthesis. Most of the currently sequenced genomes of mycobacteria possess two homologues of UvrD (UvrD1 and UvrD2, Table 1). The expression of the *uvrD* genes is upregulated upon DNA damage *in vitro*[81,100] or within macrophages.[14] A knockout of *uvrD1* in *M. smegmatis* or *M. tuberculosis* confers sensitivity to DNA damaging agents,[102,108] suggesting that *uvrD2* may not fully substitute for the function of UvrD1. Loss of UvrD1 in *M. tuberculosis* leads to a defective persistence in mouse model of infection. Biochemical characterization of UvrD1 showed that similar to *E. coli* UvrD, it has a 3′ to 5′ helicase activity[108] but it could also interact with Ku, a component of NHEJ repair pathway. Thus, the UvrD proteins of mycobacteria have multiple roles in DNA repair.

Genome sequence analysis predicts the presence of ERCC3 (XPB, common to eukaryotes) family of helicase in mycobacteria. The eukaryotic protein consists of an N terminal domain that interacts with p52 subunit, a central helicase domain and a C terminal domain. The *Mtu*XPB (Rv0861c) shares 36% identity with the human XPB, and has been cloned and biochemically characterized.[109] In addition to the helicase domain, the *Mtu*XPB contains another domain in the N-terminal. Deletion mutants of *Mtu*XPB lacking the N-terminal domain remain insoluble indicating its role in structural maintenance. The protein shows an ATP dependent 3′-5′ helicase activity and it was predicted to be essential in *M. tuberculosis*[110] by the large scale transposon mutagenesis. The *in vivo* function of this protein needs to be elucidated.

4. Concluding remarks

M. tuberculosis, often regarded as one of the most successful pathogens, survives within the host macrophages where it encounters RNI and ROS. Interestingly, this bacterium does not possess the mismatch repair system. Therefore, the other existing DNA repair pathways such as NER and BER may have a more crucial role to play in the maintenance of genomic integrity. Another important feature of this bacterium is its ability to remain latent within the host for extended periods of time. Compromised immune response of the host due to ageing, malnutrition or administration of immunosuppressant drugs leads to reactivation of bacteria. Though the mechanisms of reactivation are currently not completely understood, the bacteria should have mechanisms to safeguard its DNA and to retain its ability to multiply during reactivation. Our current understanding about the bacterial adaptation has been improved using different growth regimens to replicate various stressful conditions such as hypoxia,[111] nutritional starvation.[112] Microarray analyses from bacteria grown under such conditions have provided with the knowledge of genome-wide expression changes.[113,114] However, studies relating to DNA replication, recombination and repair under these harsh conditions are only beginning to be investigated. Recently, it has been reported that expression of DNA repair genes is upregulated when *M. smegmatis* subjected to Wayne's model of hypoxia were released into aerobic condition.[115] These observations support earlier reports,[101] wherein it was shown that expression of DNA repair genes was upregulated in *M. tuberculosis* following reactivation in a guinea pig model of infection. These results indicate that DNA repair processes may be vital for the bacteria to come out of dormancy. However, it needs to be validated if inhibition of DNA repair process hinders reactivation of bacteria. Recently, it was shown that Ung expression in *M. tuberculosis* and *M. smegmatis* decreased during growth in hypoxia.[115] Interestingly, when Ung was misexpressed in *M. smegmatis* using a hypoxia responsive promoter of *narK2* from *M. tuberculosis*, it compromised bacterial survival upon recovery under normal oxygen.[115] Presently, the impact of such misexpression of DNA repair genes in *M. tuberculosis* is not known. Such studies can be explored as possible means of generating attenuated strains.

As mentioned, many of the repair pathways are induced upon infection, and when these pathways are inactivated they resulted in reduced multiplication of mutant within the host. Molecules that target such pathways can be potential anti-TB drugs. Indeed, small chemicals that inhibit NER and render bacteria sensitive to UV have been isolated.[116] A lead compound 2-(5-amino-1,3,4-thiadiazol-2-ylbenzo[f]chromen-3-one) (ATBC) identified in a chemical screen was observed to inhibit NER activity at micro molar concentration.

Though the cytotoxic effects on human or animal cells are yet be determined, this study marks an important progress towards the possibility of using DNA repair processes as alternate drug targets. Similarly, the knockout strains deficient in one or several DNA repair pathways may offer the opportunity to generate attenuated strains.

In the recent past, the routinely used murine model of infection has come under criticism as it does not completely replicate pathological conditions of human TB. Use of alternative host such as primate model for infection has provided slightly contrasting results compared to the murine model of infection. For instance, *M. tuberculosis* strains defective in *fpg*, *nei* and *uvrB* were attenuated in a primate model of infection. In a murine model, *fpg* and *nei* mutants were not shown to have compromised growth.[52] The murine model, with its drawback[117] of not being a total replica of human TB, strains attenuated in primate model may have greater promise as vaccine candidates.

Ethical approval: Not required.

Funding: None.

Competing interests: None declared.

Acknowledgments

The work in UV's laboratory was supported by research grants from the Department of Biotechnology, New Delhi, India.

References

1. Bogdan C. Nitric oxide and the immune response. *Nat Immunol* 2001;**2**:907–16.
2. Fang FC. Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity. *J Clin Invest* 1997;**99**:2818–25.
3. Schlosser-Silverman E, Elgrably-Weiss M, Rosenshine I, Kohen R, Altuvia S. Characterization of *Escherichia coli* DNA lesions generated within J774 macrophages. *J Bacteriol* 2000;**182**:5225–30.
4. Ehrt S, Schnappinger D. Mycobacterial survival strategies in the phagosome: defence against host stresses. *Cell Microbiol* 2009;**11**:1170–8.
5. Alm RA, Ling LS, Moir DT, King BL, Brown ED, Doig PC, Smith DR, Noonan B, Guild BC, deJonge BL, Carmel G, Tummino PJ, Caruso A, Uria-Nickelsen M, Mills DM, Ives C, Gibson R, Merberg D, Mills SD, Jiang Q, Taylor DE, Vovis GF, Trust TJ. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature* 1999;**397**:176–80.
6. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, Bult CJ, Tomb JF, Dougherty BA, Merrick JM, et al. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* 1995;**269**:496–512.
7. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry 3rd CE, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 1998;**393**:537–44.
8. Ambur OH, Davidsen T, Frye SA, Balasingham SV, Lagesen K, Rognes T, Tonjum T. Genome dynamics in major bacterial pathogens. *FEMS Microbiol Rev* 2009;**33**:453–70.
9. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO global surveillance and monitoring project. *JAMA* 1999;**282**:677–86.
10. Mizrahi V, Andersen SJ. DNA repair in *Mycobacterium tuberculosis*. What have we learnt from the genome sequence? *Mol Microbiol* 1998;**29**:1331–9.
11. Davis EO, Forse LN. DNA repair: key to survival? In: Parish T, Brown A, editors. *Mycobacterium: genomics and molecular biology*. , London: Caister Academic Press; 2009. p. 79–119.
12. Springer B, Sander P, Sedlacek L, Hardt WD, Mizrahi V, Schar P, Bottger EC. Lack of mismatch correction facilitates genome evolution in mycobacteria. *Mol Microbiol* 2004;**53**:1601–9.
13. Doherty AJ, Jackson SP, Weller GR. Identification of bacterial homologues of the Ku DNA repair proteins. *FEBS Lett* 2001;**500**:186–8.
14. Della M, Palmbos PL, Tseng HM, Tonkin LM, Daley JM, Topper LM, Pitcher RS, Tomkinson AE, Wilson TE, Doherty AJ. Mycobacterial Ku and ligase proteins constitute a two-component NHEJ repair machine. *Science* 2004;**306**:683–5.
15. Rachman H, Strong M, Ulrichs T, Grode L, Schuchhardt J, Mollenkopf H, Kosmiadi GA, Eisenberg D, Kaufmann SH. Unique transcriptome signature of *Mycobacterium tuberculosis* in pulmonary tuberculosis. *Infect Immun* 2006;**74**:1233–42.
16. Kana BD, Abrahams GL, Sung N, Warner DF, Gordhan BG, Machowski EE, Tsenova L, Sacchettini JC, Stoker NG, Kaplan G, Mizrahi V. Role of the DinB homologs Rv1537 and Rv3056 in *Mycobacterium tuberculosis*. *J Bacteriol* 2010;**192**:2220–7.
17. Dos Vultos T, Mestre O, Tonjum T, Gicquel B. DNA repair in *Mycobacterium tuberculosis* revisited. *FEMS Microbiol Rev* 2009;**33**:471–87.
18. Warner DF. The role of DNA repair in *M. tuberculosis* pathogenesis. *Drug Discov Today Dis Mech* 2010;**10**:e5–11.
19. Fromme JC, Banerjee A, Verdine GL. DNA glycosylase recognition and catalysis. *Curr Opin Struct Biol* 2004;**14**:43–9.
20. Loeb LA, Preston BD. Mutagenesis by apurinic/apyrimidinic sites. *Annu Rev Genet* 1986;**20**:201–30.
21. Guillet M, Boiteux S. Endogenous DNA abasic sites cause cell death in the absence of Apn1, Apn2 and Rad1/Rad10 in Saccharomyces cerevisiae. *EMBO J* 2002;**21**:2833–41.
22. Yu SL, Lee SK, Johnson RE, Prakash L, Prakash S. The stalling of transcription at abasic sites is highly mutagenic. *Mol Cell Biol* 2003;**23**:382–8.
23. Dianov G, Price A, Lindahl T. Generation of single-nucleotide repair patches following excision of uracil residues from DNA. *Mol Cell Biol* 1992;**12**:1605–12.
24. Dianov G, Lindahl T. Reconstitution of the DNA base excision-repair pathway. *Curr Biol* 1994;**4**:1069–76.
25. Graves RJ, Felzenszwalb I, Laval J, O'Connor TR. Excision of 5'-terminal deoxyribose phosphate from damaged DNA is catalyzed by the Fpg protein of *Escherichia coli*. *J Biol Chem* 1992;**267**:14429–35.
26. Warner HR, Demple BF, Deutsch WA, Kane CM, Linn S. Apurinic/apyrimidinic endonucleases in repair of pyrimidine dimers and other lesions in DNA. *Proc Natl Acad Sci USA* 1980;**77**:4602–6.
27. Demple B, Johnson A, Fung D. Exonuclease III and endonuclease IV remove 3' blocks from DNA synthesis primers in H_2O_2-damaged *Escherichia coli*. *Proc Natl Acad Sci USA* 1986;**83**:7731–5.
28. Kumar P, Bharti SK, Varshney U. Uracil excision repair in *Mycobacterium tuberculosis* cell-free extracts. *Tuberculosis (Edinb)* 2011;**91**:212–8.
29. Friedberg EC, Walker GC, Siede W. *DNA repair and mutagenesis*. Washington, D.C.: Am Soc Microbiol; 1995.
30. Tye BK, Chien J, Lehman IR, Duncan BK, Warner HR. Uracil incorporation: a source of pulse-labeled DNA fragments in the replication of the *Escherichia coli* chromosome. *Proc Natl Acad Sci USA* 1978;**75**:233–7.
31. Vertessy BG, Toth J. Keeping uracil out of DNA: physiological role, structure and catalytic mechanism of dUTPases. *Acc Chem Res* 2009;**42**:97–106.
32. Guillet M, Van Der Kemp PA, Boiteux S. dUTPase activity is critical to maintain genetic stability in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 2006;**34**:2056–66.
33. Wang L, Weiss B. *dcd* (dCTP deaminase) gene of *Escherichia coli*: mapping, cloning, sequencing, and identification as a locus of suppressors of lethal *dut* (dUTPase) mutations. *J Bacteriol* 1992;**174**:5647–53.
34. Helt SS, Thymark M, Harris P, Aagaard C, Dietrich J, Larsen S, Willemoes M. Mechanism of dTTP inhibition of the bifunctional dCTP deaminase:dUTPase encoded by *Mycobacterium tuberculosis*. *J Mol Biol* 2008;**376**:554–69.
35. Sassetti CM, Boyd DH, Rubin EJ. Genes required for mycobacterial growth defined by high density mutagenesis. *Mol Microbiol* 2003;**48**:77–84.
36. Lindahl T, Ljungquist S, Siegert W, Nyberg B, Sperens B. DNA N-glycosidases: properties of uracil-DNA glycosidase from *Escherichia coli*. *J Biol Chem* 1977;**252**:3286–94.
37. Varshney U, Hutcheon T, van de Sande JH. Sequence analysis, expression, and conservation of *Escherichia coli* uracil DNA glycosylase and its gene (*ung*). *J Biol Chem* 1988;**263**:7776–84.
38. Slupphaug G, Mol CD, Kavli B, Arvai AS, Krokan HE, Tainer JA. A nucleotide-flipping mechanism from the structure of human uracil-DNA glycosylase bound to DNA. *Nature* 1996;**384**:87–92.
39. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. *Nature* 1995;**373**:487–93.
40. Cone R, Bonura T, Friedberg EC. Inhibitor of uracil-DNA glycosylase induced by bacteriophage PBS2. Purification and preliminary characterization. *J Biol Chem* 1980;**255**:10354–8.
41. Barrett TE, Savva R, Panayotou G, Barlow T, Brown T, Jiricny J, Pearl LH. Crystal structure of a G: T/U mismatch-specific DNA glycosylase: mismatch recognition by complementary-strand interactions. *Cell* 1998;**92**:117–29.
42. Wibley JE, Waters TR, Haushalter K, Verdine GL, Pearl LH. Structure and specificity of the vertebrate anti-mutator uracil-DNA glycosylase SMUG1. *Mol Cell* 2003;**11**:1647–59.
43. Hoseki J, Okamoto A, Masui R, Shibata T, Inoue Y, Yokoyama S, Kuramitsu S. Crystal structure of a family 4 uracil-DNA glycosylase from *Thermus thermophilus* HB8. *J Mol Biol* 2003;**333**:515–26.
44. Sartori AA, Schar P, Fitz-Gibbon S, Miller JH, Jiricny J. Biochemical characterization of uracil processing activities in the hyperthermophilic archaeon *Pyrobaculum aerophilum*. *J Biol Chem* 2001;**276**:29979–86.
45. Starkuviene V, Fritz HJ. A novel type of uracil-DNA glycosylase mediating repair of hydrolytic DNA damage in the extremely thermophilic eubacterium *Thermus thermophilus*. *Nucleic Acids Res* 2002;**30**:2097–102.
46. Pearl LH. Structure and function in the uracil-DNA glycosylase superfamily. *Mutat Res* 2000;**460**:165–81.

47. Purnapatre K, Varshney U. Uracil DNA glycosylase from *Mycobacterium smegmatis* and its distinct biochemical properties. *Eur J Biochem* 1998;**256**:580–8.
48. Kaushal PS, Talawar RK, Krishna PD, Varshney U, Vijayan M. Unique features of the structure and interactions of mycobacterial uracil-DNA glycosylase: structure of a complex of the *Mycobacterium tuberculosis* enzyme in comparison with those from other sources. *Acta Crystallogr D Biol Crystallogr* 2008;**64**:551–60.
49. Acharya N, Talawar RK, Purnapatre K, Varshney U. Use of sequence microdivergence in mycobacterial ortholog to analyze contributions of the water-activating loop histidine of *Escherichia coli* uracil-DNA glycosylase in reactant binding and catalysis. *Biochem Biophys Res Commun* 2004;**320**:893–9.
50. Venkatesh J, Kumar P, Krishna PS, Manjunath R, Varshney U. Importance of uracil DNA glycosylase in *Pseudomonas aeruginosa* and *Mycobacterium smegmatis*, G+C-rich bacteria, in mutation prevention, tolerance to acidified nitrite, and endurance in mouse macrophages. *J Biol Chem* 2003;**278**:24350–8.
51. Kurthkoti K, Kumar P, Jain R, Varshney U. Important role of the nucleotide excision repair pathway in *Mycobacterium smegmatis* in conferring protection against commonly encountered DNA-damaging agents. *Microbiology* 2008;**154**:2776–85.
52. Sassetti CM, Rubin EJ. Genetic requirements for mycobacterial survival during infection. *Proc Natl Acad Sci U S A* 2003;**100**:12989–94.
53. Handa P, Acharya N, Varshney U. Chimeras between single-stranded DNA-binding proteins from *Escherichia coli* and *Mycobacterium tuberculosis* reveal that their C-terminal domains interact with uracil DNA glycosylases. *J Biol Chem* 2001;**276**:16992–7.
54. Acharya N, Varshney U. Biochemical properties of single-stranded DNA-binding protein from *Mycobacterium smegmatis*, a fast-growing mycobacterium and its physical and functional interaction with uracil DNA glycosylases. *J Mol Biol* 2002;**318**:1251–64.
55. Sartori AA, Fitz-Gibbon S, Yang H, Miller JH, Jiricny J. A novel uracil-DNA glycosylase with broad substrate specificity and an unusual active site. *EMBO J* 2002;**21**:3182–91.
56. Srinath T, Bharti SK, Varshney U. Substrate specificities and functional characterization of a thermo-tolerant uracil DNA glycosylase (UdgB) from *Mycobacterium tuberculosis*. *DNA Repair (Amst)* 2007;**6**:1517–28.
57. Parikh SS, Mol CD, Slupphaug G, Bharati S, Krokan HE, Tainer JA. Base excision repair initiation revealed by crystal structures and binding kinetics of human uracil-DNA glycosylase with DNA. *EMBO J* 1998;**17**:5214–26.
58. Bharti SK, Varshney U. Analysis of the impact of a uracil DNA glycosylase attenuated in AP-DNA binding in maintenance of the genomic integrity in *Escherichia coli*. *Nucleic Acids Res* 2010;**38**:2291–301.
59. Wanner RM, Castor D, Guthlein C, Bottger EC, Springer B, Jiricny J. The uracil DNA glycosylase UdgB of *Mycobacterium smegmatis* protects the organism from the mutagenic effects of cytosine and adenine deamination. *J Bacteriol* 2009;**191**:6312–9.
60. Malshetty VS, Jain R, Srinath T, Kurthkoti K, Varshney U. Synergistic effects of UdgB and Ung in mutation prevention and protection against commonly encountered DNA damaging agents in *Mycobacterium smegmatis*. *Microbiology* 2010;**156**:940–9.
61. Lu AL, Li X, Gu Y, Wright PM, Chang DY. Repair of oxidative DNA damage: mechanisms and functions. *Cell Biochem Biophys* 2001;**35**:141–70.
62. David SS, O'Shea VL, Kundu S. Base-excision repair of oxidative DNA damage. *Nature* 2007;**447**:941–50.
63. Jain R, Kumar P, Varshney U. A distinct role of formamidopyrimidine DNA glycosylase (MutM) in down-regulation of accumulation of G, C mutations and protection against oxidative stress in mycobacteria. *DNA Repair (Amst)* 2007;**6**:1774–85.
64. Olsen I, Balasingham SV, Davidsen T, Debebe E, Rodland EA, van Soolingen D, Kremer K, Alseth I, Tonjum T. Characterization of the major formamidopyrimidine-DNA glycosylase homolog in *Mycobacterium tuberculosis* and its linkage to variable tandem repeats. *FEMS Immunol Med Microbiol* 2009;**56**:151–61.
65. Guo Y, Bandaru V, Jaruga P, Zhao X, Burrows CJ, Iwai S, Dizdaroglu M, Bond JP, Wallace SS. The oxidative DNA glycosylases of *Mycobacterium tuberculosis* exhibit different substrate preferences from their *Escherichia coli* counterparts. *DNA Repair (Amst)* 2010;**9**:177–90.
66. Sidorenko VS, Rot MA, Filipenko ML, Nevinsky GA, Zharkov DO. Novel DNA glycosylases from *Mycobacterium tuberculosis*. *Biochemistry (Mosc)* 2008;**73**:442–50.
67. Au KG, Clark S, Miller JH, Modrich P. *Escherichia coli* mutY gene encodes an adenine glycosylase active on G-A mispairs. *Proc Natl Acad Sci USA* 1989;**86**:8877–81.
68. Tsai-Wu JJ, Liu HF, Lu AL. *Escherichia coli* MutY protein has both N-glycosylase and apurinic/apyrimidinic endonuclease activities on A: C and A: G mispairs. *Proc Natl Acad Sci U S A* 1992;**89**:8779–83.
69. Zhang QM, Ishikawa N, Nakahara T, Yonei S. *Escherichia coli* MutY protein has a guanine-DNA glycosylase that acts on 7,8-dihydro-8-oxoguanine:guanine mispair to prevent spontaneous G: C–>C: G transversions. *Nucleic Acids Res* 1998;**26**:4669–75.
70. Noll DM, Gogos A, Granek JA, Clarke ND. The C-terminal domain of the adenine-DNA glycosylase MutY confers specificity for 8-oxoguanine.adenine mispairs and may have evolved from MutT, an 8-oxo-dGTPase. *Biochemistry* 1999;**38**:6374–9.
71. Volk DE, House PG, Thiviyanathan V, Luxon BA, Zhang S, Lloyd RS, Gorenstein DG. Structural similarities between MutT and the C-terminal domain of MutY. *Biochemistry* 2000;**39**:7331–6.
72. Kurthkoti K, Srinath T, Kumar P, Malshetty VS, Sang PB, Jain R, Manjunath R, Varshney U. A distinct physiological role of MutY in mutation prevention in mycobacteria. *Microbiology* 2010;**156**:88–93.
73. Shimizu M, Gruz P, Kamiya H, Kim SR, Pisani FM, Masutani C, Kanke Y, Harashima H, Hanaoka F, Nohmi T. Erroneous incorporation of oxidized DNA precursors by Y-family DNA polymerases. *EMBO Rep* 2003;**4**:269–73.
74. Dutta NK, Mehra S, Didier PJ, Roy CJ, Doyle LA, Alvarez X, Ratterree M, Be NA, Lamichhane G, Jain SK, Lacey MR, Lackner AA, Kaushal D. Genetic requirements for the survival of tubercle bacilli in primates. *J Infect Dis* 2010;**201**:1743–52.
75. Maki H, Sekiguchi M. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature* 1992;**355**:273–5.
76. Dos Vultos T, Blazquez J, Rauzier J, Matic I, Gicquel B. Identification of Nudix hydrolase family members with an antimutator role in *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. *J Bacteriol* 2006;**188**:3159–61.
77. Lari N, Rindi L, Bonanni D, Tortoli E, Garzelli C. Mutations in *mutT* genes of *Mycobacterium tuberculosis* isolates of Beijing genotype. *J Med Microbiol* 2006;**55**:599–603.
78. Moreland NJ, Charlier C, Dingley AJ, Baker EN, Lott JS. Making sense of a missense mutation: characterization of MutT2, a Nudix hydrolase from *Mycobacterium tuberculosis*, and the G58R mutant encoded in W-Beijing strains of *M. tuberculosis*. *Biochemistry* 2009;**48**:699–708.
79. Lu LD, Sun Q, Fan XY, Zhong Y, Yao YF, Zhao GP. Mycobacterial MazG is a novel NTP pyrophosphohydrolase involved in oxidative stress response. *J Biol Chem* 2010;**285**:28076–85.
80. Bjelland S, Bjoras M, Seeberg E. Excision of 3-methylguanine from alkylated DNA by 3-methyladenine DNA glycosylase I of *Escherichia coli*. *Nucleic Acids Res* 1993;**21**:2045–9.
81. Boshoff HI, Reed MB, Barry 3rd CE, Mizrahi V. DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in *Mycobacterium tuberculosis*. *Cell* 2003;**113**:183–93.
82. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, Schoolnik GK. Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: insights into the Phagosomal environment. *J Exp Med* 2003;**198**:693–704.
83. Durbach SI, Springer B, Machowski EE, North RJ, Papavinasasundaram KG, Colston MJ, Bottger EC, Mizrahi V. DNA alkylation damage as a sensor of nitrosative stress in *Mycobacterium tuberculosis*. *Infect Immun* 2003;**71**:997–1000.
84. Yang M, Aamodt RM, Dalhus B, Balasingham S, Helle I, Andersen P, Tønjum T, Alseth I, Rognes T, Bjørås M. The ada operon of *Mycobacterium tuberculosis* encodes two DNA methyltransferases for inducible repair of DNA alkylation damage. *DNA Repair*; 2011; doi:10.1016/j.dnarep.2011.03.007.
85. Boiteux S, Guillet M. Abasic sites in DNA: repair and biological consequences in *Saccharomyces cerevisiae*. *DNA Repair (Amst)* 2004;**3**:1–12.
86. Kuo CF, Mol CD, Thayer MM, Cunningham RP, Tainer JA. Structure and function of the DNA repair enzyme exonuclease III from *E. coli*. *Ann N Y Acad Sci* 1994;**726**:223–34. discussion 34–5.
87. Suvarnapunya AE, Lagassé HA, Stein MA. The role of DNA base excision repair in the pathogenesis of *Salmonella enterica* serovar Typhimurium. *Mol Microbiol* 2003;**48**(2):549–59.
88. Hanawalt PC, Cooper PK, Ganesan AK, Smith CA. DNA repair in bacteria and mammalian cells. *Annu Rev Biochem* 1979;**48**:783–836.
89. Friedberg EC, Bridges BA. *Cellular responses to DNA damage*. New York: Alan R. Liss, Inc; 1983.
90. Sancar A, Rupp WD. A novel repair enzyme: UVRABC excision nuclease of *Escherichia coli* cuts a DNA strand on both sides of the damaged region. *Cell* 1983;**33**:249–60.
91. Huang JC, Svoboda DL, Reardon JT, Sancar A. Human nucleotide excision nuclease removes thymine dimers from DNA by incising the 22nd phosphodiester bond 5' and the 6th phosphodiester bond 3' to the photodimer. *Proc Natl Acad Sci USA* 1992;**89**:3664–8.
92. Snowden A, Kow YW, Van Houten B. Damage repertoire of the *Escherichia coli* UvrABC nuclease complex includes abasic sites, base-damage analogues, and lesions containing adjacent 5' or 3' nicks. *Biochemistry* 1990;**29**:7251–9.
93. Van Houten B, Snowden A. Mechanism of action of the *Escherichia coli* UvrABC nuclease: clues to the damage recognition problem. *Bioessays* 1993;**15**:51–9.
94. Verhoeven EE, Wyman C, Moolenaar GF, Goosen N. The presence of two UvrB subunits in the UvrAB complex ensures damage detection in both DNA strands. *EMBO J* 2002;**21**:4196–205.
95. Graham JE, Clark-Curtiss JE. Identification of *Mycobacterium tuberculosis* RNAs synthesized in response to phagocytosis by human macrophages by selective capture of transcribed sequences (SCOTS). *Proc Natl Acad Sci U S A* 1999;**96**:11554–9.
96. Darwin KH, Ehrt S, Gutierrez-Ramos JC, Weich N, Nathan CF. The proteasome of *Mycobacterium tuberculosis* is required for resistance to nitric oxide. *Science* 2003;**302**:1963–6.
97. Darwin KH, Nathan CF. Role for nucleotide excision repair in virulence of *Mycobacterium Tuberculosis*. *Infect Immun* 2005;**73**:4581–7.
98. Rangarajan S, Woodgate R, Goodman MF. Replication restart in UV-irradiated *Escherichia coli* involving pols II, III, V, PriA, RecA and RecFOR proteins. *Mol Microbiol* 2002;**43**:617–28.
99. Moolenaar GF, van Rossum-Fikkert S, van Kesteren M, Goosen N. Cho, a second endonuclease involved in *Escherichia coli* nucleotide excision repair. *Proc Natl Acad Sci USA* 2002;**99**:1467–72.

100. Rand L, Hinds J, Springer B, Sander P, Buxton RS, Davis EO. The majority of inducible DNA repair genes in *Mycobacterium tuberculosis* are induced independently of RecA. *Mol Microbiol* 2003;**50**:1031–42.
101. Kesavan AK, Brooks M, Tufariello J, Chan J, Manabe YC. Tuberculosis genes expressed during persistence and reactivation in the resistant rabbit model. *Tuberculosis (Edinb)* 2009;**89**:17–21.
102. Guthlein C, Wanner RM, Sander P, Davis EO, Bosshard M, Jiricny J, Bottger EC, Springer B. Characterization of the mycobacterial NER system reveals novel functions of the *uvrD1* helicase. *J Bacteriol* 2009;**191**:555–62.
103. Mellon I, Hanawalt PC. Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature* 1989;**342**:95–8.
104. Selby CP, Sancar A. Gene- and strand-specific repair in vitro: partial purification of a transcription-repair coupling factor. *Proc Natl Acad Sci USA* 1991;**88**:8232–6.
105. Cappelli G, Volpe E, Grassi M, Liseo B, Colizzi V, Mariani F. Profiling of *Mycobacterium tuberculosis* gene expression during human macrophage infection: upregulation of the alternative sigma factor G, a group of transcriptional regulators, and proteins with unknown function. *Res Microbiol* 2006;**157**:445–55.
106. Prabha S, Rao DN, Nagaraja V. Distinct properties of hexameric but Functionally conserved *Mycobacterium tuberculosis* transcription-repair coupling factor. *PLoS ONE* 2011;**6**:e19131. doi:10.1371/journal.pone.0019131.
107. Han J, Sahin O, Barton YW, Zhang Q. Key role of Mfd in the development of fluoroquinolone resistance in *Campylobacter jejuni*. *PLoS Pathog* 2008;**4**. e1000083.
108. Sinha KM, Stephanou NC, Gao F, Glickman MS, Shuman S. Mycobacterial UvrD1 is a Ku-dependent DNA helicase that plays a role in multiple DNA repair events, including double-strand break repair. *J Biol Chem* 2007;**282**: 15114–25.
109. Biswas T, Pero JM, Joseph CG, Tsodikov OV. DNA-dependent ATPase activity of bacterial XPB helicases. *Biochemistry* 2009;**48**:2839–48.
110. Lamichhane G, Zignol M, Blades NJ, Geiman DE, Dougherty A, Grosset J, Broman KW, Bishai WR. A postgenomic method for predicting essential genes at subsaturation levels of mutagenesis: application to *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 2003;**100**:7213–8.
111. Wayne LG, Hayes LG. An in vitro model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. *Infect Immun* 1996;**64**:2062–9.
112. Betts JC, Lukey PT, Robb LC, McAdam RA, Duncan K. Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. *Mol Microbiol* 2002;**43**:717–31.
113. Muttucumaru DG, Roberts G, Hinds J, Stabler RA, Parish T. Gene expression profile of *Mycobacterium tuberculosis* in a non-replicating state. *Tuberculosis (Edinb)* 2004;**84**:239–46.
114. Hampshire T, Soneji S, Bacon J, James BW, Hinds J, Laing K, Stabler RA, Marsh PD, Butcher PD. Stationary phase gene expression of *Mycobacterium tuberculosis* following a progressive nutrient depletion: a model for persistent organisms? *Tuberculosis (Edinb)* 2004;**84**:228–38.
115. Kurthkoti K, Varshney U. Detrimental effects of hypoxia-specific expression of uracil DNA glycosylase (Ung) in *Mycobacterium smegmatis*. *J Bacteriol* 2010;**192**:6439–46.
116. Mazloum N, Stegman MA, Croteau DL, Van Houten B, Kwon NS, Ling Y, Dickinson C, Venugopal A, Towheed MA, Nathan C. Identification of a chemical that inhibits the mycobacterial UvrABC complex in nucleotide excision repair. *Biochemistry* 2011;**50**:1329–35.
117. Rhoades ER, Frank AA, Orme IM. Progression of chronic pulmonary tuberculosis in mice aerogenically infected with virulent *Mycobacterium tuberculosis*. *Tuber Lung Dis* 1997;**78**:57–66.
118. Sinha KM, Glickman MS, Shuman S. Mutational analysis of mycobacterium UvrD1 identifies functional groups required for ATP hydrolysis, DNA unwinding, and chemomechanical coupling. *Biochemistry* 2009;**48**:4019–30.
119. Sinha KM, Stephanou NC, Unciuleac MC, Glickman MS, Shuman S. Domain requirements for DNA unwinding by mycobacterial UvrD2, an essential DNA helicase. *Biochemistry* 2008;**47**:9355–64.
120. Curti E, Smerdon SJ, Davis EO. Characterization of the helicase activity and substrate specificity of *Mycobacterium tuberculosis* UvrD. *J Bacteriol* 2007;**189**:1542–55.
121. Gordhan BG, Andersen SJ, De Meyer AR, Mizrahi V. Construction by homologous recombination and phenotypic characterization of a DNA polymerase domain *polA* mutant of *Mycobacterium smegmatis*. *Gene* 1996;**178**:125–30.
122. Huberts P, Mizrahi V. Cloning and sequence analysis of the gene encoding the DNA polymerase I from *Mycobacterium tuberculosis*. *Gene* 1995;**164**:133–6.
123. Gong C, Martins A, Bongiorno P, Glickman M, Shuman S. Biochemical and genetic analysis of the four DNA ligases of mycobacteria. *J Biol Chem* 2004;**279**:20594–606.