



■ R E V I E W

# DNA repair systems and the pathogenesis of *Mycobacterium tuberculosis*: varying activities at different stages of infection

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## A B S T R A C T

*Mycobacteria*, including most of all MTB (*Mycobacterium tuberculosis*), cause pathogenic infections in humans and, during the infectious process, are exposed to a range of environmental insults, including the host's immune response. From the moment MTB is exhaled by infected individuals, through an active and latent phase in the body of the new host, until the time they reach the reactivation stage, MTB is exposed to many types of DNA-damaging agents. Like all cellular organisms, MTB has efficient DNA repair systems, and these are believed to play essential roles in mycobacterial pathogenesis. As different stages of infection have great variation in the conditions in which mycobacteria reside, it is possible that different repair systems are essential for progression to specific phases of infection. MTB possesses homologues of DNA repair systems that are found widely in other species of bacteria, such as nucleotide excision repair, base excision repair and repair by homologous recombination. MTB also possesses a system for non-homologous end-joining of DNA breaks, which appears to be widespread in prokaryotes, although its presence is sporadic within different species within a genus. However, MTB does not possess homologues of the typical mismatch repair system that is found in most bacteria. Recent studies have demonstrated that DNA repair genes are expressed differentially at each stage of infection. In the present review, we focus on different DNA repair systems from mycobacteria and identify questions that remain in our understanding of how these systems have an impact upon the infection processes of these important pathogens.

## INTRODUCTION

MTB (*Mycobacterium tuberculosis*), the causative agent of TB (tuberculosis), is a micro-organism that is among the greatest 'enemies' of humanity. According to a WHO (World Health Organization) report from 2009 [1], more

than 2 billion people are currently infected with TB bacilli and 1 in 10 of infected people will become sick with active TB during their lifetime. Indeed, in 2008, 1.8 million of people died from TB [1]. Even though several anti-mycobacterial drugs have been discovered and are currently in use, the results of the applied

**Key words:** aerosol droplet, DNA repair, drug susceptibility infection, macrophage, *Mycobacterium tuberculosis*, granuloma, mutation rate.

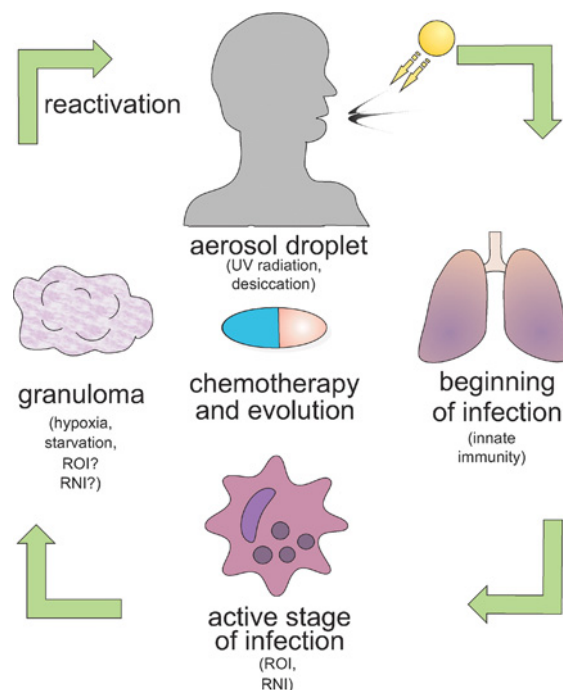
**Abbreviations:** BCG, *Mycobacterium bovis* BCG; BER, base excision repair; DSB, double-strand break; HR, homologous recombination; MDR, multi-drug resistant; MMR, mismatch repair; Ms, *Mycobacterium smegmatis*; MTB, *Mycobacterium tuberculosis*; NER, nucleotide excision repair; NHEJ, non-homologous end-joining; NOS, NO synthase; ONOO<sup>-</sup>, peroxynitrite; RNI, reactive nitrogen intermediate; ROI, reactive oxygen intermediate; SNP, single nucleotide polymorphism; SP, surfactant protein; TB, tuberculosis; XDR, extensively drug-resistant.

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chemotherapy are far from satisfactory. Treatment requires many months of taking several drugs and there are many side effects. The drugs are, in some cases, difficult to obtain and therapeutic regimes are difficult to follow. When coupled with the nature of MTB's infectious process itself, this has resulted in widespread emergence of drug-resistant and multidrug-resistant strains of MTB. Therefore it is of great importance to understand how MTB succeeds in infecting its host, as such advances in knowledge will help the therapeutic fight against this pathogen. Proteins involved in DNA metabolism offer interesting possibilities as drug targets, because many of them provide essential functions to bacteria and, in many cases, are distinct from human proteins at the biochemical and structural level.

The infectious process of MTB can be divided into several stages [2]. Infection begins when bacteria are exhaled by infected individuals and are transported in aerosol droplets through the air. Small numbers of bacteria are then inhaled by healthy individuals and settle as a primary infection that inhabits macrophages. At this point, depending on the efficiency of the host's immune response, bacteria may be eradicated from the host organism. However, if the host's response is insufficient, macrophage-residing mycobacteria inhibit the fusion of phagosomes and lysosomes and, following the primary infection stage, may enter a stage of latency in the form of a granuloma. Several types of cells are recruited to the site of infection, including lymphocytes, monocytes and neutrophils, and they cluster together to form a closed environment where the mycobacteria may reside for decades. MTB resides in a state of latency until the moment when there is the breakage of the granuloma and reactivation of the disease (Figure 1). Important details about each phase of the infection process will be discussed below.

As mycobacteria are exposed constantly to hostile environments, which, among other things, lead to lesions and modifications of the bacterial DNA, the integrity of its genome is protected by mechanisms of DNA repair [3]. These mechanisms are important to consider in the context of mycobacteria because they could influence processes leading to drug resistance and they may offer novel targets for antibiotics [4]. Mycobacteria possess DNA repair systems common to prokaryotes, such as HR (homologous recombination), BER (base excision repair) and NER (nucleotide excision repair). Mycobacteria also possess a system for NHEJ (non-homologous end-joining) of DNA breaks, which is widespread in prokaryotes, although its presence is sporadic within different genera of bacteria. However, in contrast with many other prokaryotes, mycobacteria do not possess mechanisms known to be required for MMR (mismatch repair) [5]. A description of the specific roles of each protein involved in DNA repair exceeds the space available within the present review; however,



**Figure 1** Overview of the MTB infection process in humans

Infection of humans by MTB occurs via several distinct stages. At each stage of infection, the bacteria are exposed to various types of stress that may induce different types of DNA damage.

several recent reviews have covered mycobacterial DNA repair proteins [4,6,7].

Microarray analysis has revealed that almost half of the MTB DNA repair genes are continuously expressed during exponential phase growth in broth, suggesting that the bacteria have to continuously counteract the constant exposure to DNA-damaging agents [8] (see Supplementary Table S1 at <http://www.clinsci.org/cs/119/cs1190187add.htm>). Intriguingly, only a few of the genes associated with DNA repair are required for optimal growth of MTB in broth, including Rv2554c, *dut*, *ligA*, *polA*, *adnB* and those encoding the NER-related proteins UvrD1, UvrD2 and UvrC [9] (see Supplementary Table S1). In fact *uvrD2* [10] and *ligA* [11] have been shown to be essential for the survival of *Ms* (*M. smegmatis*), a non-pathogenic saprophytic bacteria found mostly in soil, water or plants worldwide and used as a genetic model for the *Mycobacterium* genus. Another MTB study identified that survival *in vivo* in mice required three BER proteins (Ung, Nfo/End and XthA), MazG and RecN [12]. Most of the MTB DNA repair genes have been shown to be non-essential for survival in broth, but the growth of a strain defective in *recA* was slowed down [9] (see Supplementary Table S1). Independent of the probable constitutive expression of DNA repair genes, their expression changes in response to different environments encountered during the process

of infection. Thus, as we discuss below, *in vitro* and *in vivo* studies provide us with an insight into the role of DNA repair proteins in the pathogenesis of MTB at different stages of infection. For more information see Supplementary Table S1.

## AEROSOL DROPLETS

MTB is transmitted in aerosol droplets, which are coughed out by infected individuals. The size distribution of the aerosol droplets is variable, but most particle sizes are within the size that can be taken into the respiratory system [13]. Experiments performed in 1969 reported that the rate of survival of mycobacteria in aerosol droplets falls to 50% after 6 h [14]. More recent findings have shown that, if MTB is held for 5 min in an aerosol of artificial saliva, its viability declines to 55%, after 30 min less than 10% remain viable and after 1 h the percentage of viable cells is close to zero [15]. The authors of that study point out that the viability of MTB might actually be further compromised because of another factor influencing survival of mycobacteria in the natural environment, namely UV radiation. In fact UV radiation, apart from being present in the natural environment, is a known agent for sterilizing mycobacteria-contaminated air [16–18]. Environmental studies highlight the importance of both factors in the survival of mycobacteria; for example, lower transmission rates of TB are observed at higher altitudes, where humidity is low and the exposure to UV light is stronger [19]. Studies based on artificially generated droplets carrying BCG (*M. bovis* BCG), the causative agent of TB in cattle, show a link between both factors: increased UV resistance is observed at high relative humidity [20]. Nevertheless, it is clear that the survival rate of mycobacteria is drastically reduced when they remain in the aerosol droplet, especially if it is exposed to environmental conditions such as desiccation and UV irradiation.

It is known that both desiccation and UV radiation lead to DNA damage. Intracellular dehydration leads to DSBs (double-strand breaks) of DNA, but also DNA conformation changes and, as a result, a different susceptibility to UV-induced DNA damage [21]. UV exposure leads to cyclobutane pyrimidine dimers and pyrimidine(6–4)pyrimidine photoproducts, in which two adjacent pyrimidines are covalently linked. It also leads to some damage of bases, producing cytosine hydrate and thymine glycol. Thus it is clear that, in order to survive the ‘outside host’ environmental stage of their life cycle, mycobacteria must employ DNA repair systems. However, currently it remains uncertain whether DNA repair is active while the mycobacteria are still suspended in aerosol droplets or when they inhabit their new host.

Desiccation of mycobacteria leads to DSBs in their DNA and, if unrepaired, these can be lethal to the cells in which they occur. There are two systems in mycobacteria known to repair this kind of DNA damage: HR and NHEJ. Construction of mutant strains of Ms lacking the proteins RecA (for HR) and Ku and/or LigD (for NHEJ) showed that neither system of DSB repair is essential for growth *in vitro* [11]. However, both systems are required to maintain the viability of Ms under desiccation in the broth culture (Table 1) and, interestingly, the susceptibility to desiccation of the mutant lacking both HR and NHEJ did not vary from the susceptibility of strains that were defective for only NHEJ or HR [22]. Whether such an outcome is a result of an interplay between NHEJ and HR while repairing DSBs, or the susceptibility of the RecA mutant is due to the disruption of other cellular pathways, such as the SOS response, is still unknown. Notably, differences in the environmental niches of Ms and MTB must be taken into consideration when assessing these data.

The core mechanism for removal and repair of UV-induced damage is NER. It consists of the UvrABC nuclease enzyme complex and the UvrD helicase. Mutations that influence NER, including *polA* of Ms [23], *uvrB* for both Ms [24] and MTB [25] and *uvrD1* of Ms [26], lead to increased susceptibility to UV radiation *in vitro* (Table 1). Interestingly, the combined deletion of both *uvrB* and *uvrD1* of Ms has an additive effect on the survival rate of mycobacteria under UV radiation [26]. Such a result implies an involvement for both gene products in NER, but also suggests that one of the proteins has an additional role in another DNA repair pathway. Which other repair systems could be involved in UV damage repair? During broth growth, UvrD1 has been reported to be an interaction partner for Ku protein, one of the major proteins in NHEJ. Nevertheless, UvrD1 function in UV damage repair seems to be independent of Ku, as a Ku-deficient strain of Ms displays wild-type sensitivity to UV radiation [27,28].

Another type of DNA metabolism that is important for the repair of UV-induced damage *in vitro* is HR. MTB RecA is induced in Ms broth culture following UV exposure [29], and mutation in *recA* makes this Ms mutant increasingly susceptible to UV irradiation [26,30]. However, neither the effect of a double mutation in NER and HR under UV-induced stress nor the relationship between HR and NER proteins in mycobacteria is completely understood.

Microarray analysis of MTB genes induced under UV treatment in broth cultures confirmed the role of HR and NER in the repair of such damage. It also highlighted several other possible proteins that could be involved in such repair, including Ogt, Ada/AlkA, DinF, Lhr, DinX and DnaE2 [31,32]. It has to be underlined, though, that two independent studies did not produce fully consistent results [31,32] (see Table 1). Another puzzling observation

**Table 1** DNA repair genes affected by UV or desiccation

All results are for MTB unless otherwise stated, and are from *in vitro* studies, rather than aerosol droplet studies. +, Sensitivity or overexpression; \*, at least a 2-fold change in expression level.

Locus	Gene	Induced by UV in MTB [31]*	Induced by UV in MTB [32]	Mutants sensitive to UV exposure	Mutants sensitive to desiccation in Ms [22]
Rv0937c	<i>ku</i>				+
Rv0938	<i>ligD</i>				+
Rv0949	<i>uvrD1</i>			Ms + [26]	
Rv1316c	<i>ogt</i>		+		
Rv1317c	<i>ada, alkA</i>		+		
Rv1629	<i>polA</i>			Ms + [23]	
Rv1633	<i>uvrB</i>		+	+ [25]; Ms + [24]	
Rv1638	<i>uvrA</i>		+		
Rv2593c	<i>ruvA</i>		+		
Rv2594c	<i>ruvC</i>	+	+		
Rv2697c	<i>dut</i>				
Rv2719c		+			
Rv2720	<i>lexA</i>		+		
Rv2736c	<i>recX</i>	+	+		
Rv2737c	<i>recA</i>	+	+	Ms + [26,30]	+
Rv3198c	<i>uvrD2</i>		+		
Rv3202c	<i>adnA</i>		+		
Rv3296	<i>lhr</i>		+		
Rv3370c	<i>dnaE2</i>		+		
Rv3394c	<i>dinX</i>		+		
Rv3395c	<i>dinF</i>	+			
Rv3585	<i>radA</i>		+		

relates to the presence of MTB KatG catalase-peroxidase with resistance to UV damage, since an *Escherichia coli* *recA*-deficient strain carrying MTB *katG* had higher survival rates under exposure to UV radiation in broth culture [33]. The observed phenomenon was not due simply to catalase-peroxidase activity. On the other hand such an effect was not observed in tubercle bacilli clinical strains [33]. However, *katG* is induced by UV radiation, as identified in broth culture analysis of MTB [31].

## MACROPHAGE INFECTION

The second stage of infection by mycobacteria starts as soon as they are inhaled by a healthy individual. This stage proceeds by the bacteria inhabiting and residing in macrophages, eventually forming a granuloma. From this point on the mycobacteria are continuously exposed to both oxidative and nitrosative stress generated by the activated macrophages that they inhabit [34].

Macrophages produce ROIs (reactive oxygen intermediates) and RNIs (reactive nitrogen intermediates) as cytotoxic agents in the immune response to pathogens. Production of these compounds by host cells appears to be essential in the control of mycobacterial infection, as murine mutants deficient in NOS (NO synthase)

responsible for producing RNIs [34–36] or phagocyte oxidase cytochrome *b* responsible for producing ROIs [34] experience exaggerated mycobacterial infection. The mutagenic effect and cytotoxic effect of ROIs and RNIs is based on several chemical reactions leading to DNA damage. NO can undergo auto-oxidation to form N<sub>2</sub>O<sub>3</sub> (nitrous anhydride), which can nitrosinate amines to N-nitrosamines, which, after activation, alkylate DNA through an indirect mechanism [37]. Direct nitrosation of DNA bases leads to the formation of diazonium ions and subsequent deamination, which can result in base transitions, transversions and cross-links. Moreover, deamination of guanine leads to the formation of xanthine, which is unstable in DNA and can depurinate easily, leaving an abasic site that can be cleaved by endonucleases to generate single strand breaks. NO can also react with superoxide giving rise to ONOO<sup>−</sup> (peroxynitrite), a compound which causes more severe DNA damage at smaller doses than NO. ONOO<sup>−</sup> can nitrate DNA, resulting in the damage described above, but it can also oxidize it [37]. Even though oxidation can occur on all four bases of DNA, the most common DNA lesion that is used as a marker of oxidative stress is the formation of 8-hydroxyguanine [38]. Oxidized bases can sometimes be pro-mutagenic and, since some can block replication forks, they can be lethal [39].

One of the first lines of defence against pathogens invading the respiratory tract is the surfactant of the lungs. Within the fluid, SPs (surfactant proteins) are responsible for interacting with incoming pathogens. Of particular interest, SP-A increases phagocytosis of BCG [40] and, while in complex with BCG, has been shown to stimulate expression of NOS by rat alveolar macrophages [41]. However, contact with surfactant itself, without the presence of macrophages, seems to have a minor effect on the expression of DNA repair genes in MTB. Results from a transcriptional analysis of the response of MTB to bovine lung surfactant show that only one DNA repair gene, *mutY*, was up-regulated upon contact with bovine lung surfactant, whereas a few, including *nth*, *Rv1160*, *Rv0944* and *ercc3*, were down-regulated [42].

Several studies have confirmed the role of mycobacterial proteins involved in NER and BER in the removal of damage encountered in macrophages (Table 2). Interestingly, the MTB gene *ada/alkA* was up-regulated in both human [43] and murine [44] macrophages and up-regulated in response to  $H_2O_2$  in broth culture [32,44]. Furthermore, MTB strains deficient in *ada/alkA* had increased susceptibility to RNIs, but the gene seems to be irrelevant for MTB during survival in mice, as the knockout mutant had a wild-type survival rate [45]. Further interesting observations have been made in relation to *uvrB*, which is required for optimal growth of MTB in mice [25]. Two studies showed, using different reagents and methods, that Ms with *uvrB* knocked out were more susceptible to ROIs than the wild-type in broth culture [24,26]. However, experiments in MTB, performed using a disc diffusion assay on agar plates [25] and in broth cultures [45a] but performed with slight modifications of the methods in relation to the studies performed in Ms, produced contradictory results. The differences in the results obtained in Ms and MTB may be a result of the different approaches used by the authors; however, deletion of *uvrB* in both MTB and Ms increased their susceptibility to RNIs in broth cultures (Table 2).

Microarray experiments suggest that HR genes are expressed during the active phase of infection (Table 2). MTB genes involved in this type of repair were up-regulated within murine macrophages or in response to  $H_2O_2$  [44] and also in human macrophages [46]. However, microarray experiments following phagocytosis by human and murine macrophages suggest possible differences between the macrophage environments of the two species. The pattern of gene expression in both studies was different, most notably seen in the case of *recX*, as it was up-regulated in murine macrophages [44,47], but down-regulated after 4 h of human macrophage infection [43] (Table 2).

Studies have reported that expression of the NHEJ major proteins is unaltered in response to the environment encountered in macrophages. However, since the core NHEJ proteins are constitutively expressed

in MTB during exponential growth in the broth culture [8], NHEJ might still play an important role at this stage of infection. Taken together, the combined results suggest that DSBs occurring in mycobacteria at this stage of infection might be preferentially repaired by HR. However, it is notable that a *recA*-deficient mutant of BCG does not influence the bacteria's rate of survival *in vivo* in a mouse model, even though it shows increased susceptibility to DNA alkylating agents *in vitro* [48]. Thus there is still much to learn as to whether DSBs within the mycobacterial genome influence the infection of macrophages. For more information regarding the DNA repair gene response during the active stage of infection of MTB, see Table 2.

## GRANULOMA

After the active phase of infection is over, mycobacteria persist in the infected lung and settle in to cause a chronic infection. Constant antigenic stimulation and accumulation of T-cells leads to the formation of a granuloma, which can maintain viable bacilli for decades.

The architecture and environment of granulomas vary between different organisms [49]. The structure of a human granuloma is characterized by a central region of large CD68<sup>+</sup> epitheloid cells, surrounded by macrophages, T-lymphocytes (predominantly CD4<sup>+</sup>) and multi-nucleate giant Langerhans cells. In mice there is a co-location of CD4<sup>+</sup> T-cells and macrophages, some of which differentiate into epitheloid cells, but the giant Langerhans cells are absent. In both organisms there is the presence of B-lymphocyte aggregates, but their spatial relationship with other immune cells is different. Another distinctive feature of human granulomas is caseous necrosis, which is also absent in the murine version. This is a central acellular eosinophilic region of granuloma formed as a result of cells undergoing necrosis and/or apoptosis under intense cytokine and direct cell-cell activation. The central region of the granuloma does not contain blood vessels and becomes hypoxic [49], a feature not observed in the murine model [50]. Hypoxia then further increases the chances for cells to undergo necrosis [49]. As the granuloma becomes necrotic, it may either be resolved by fibrosis and calcification or become liquefied, therefore leading to dissemination of any pathogens contained within it. Details about granuloma formation and deconstruction have been reviewed previously [51].

There is still an ongoing discussion as to whether MTBs undergo cell division when they are inside granulomatous tissue [52,53]. Nevertheless, it seems that MTBs have varying gene expression within granuloma. An important aspect in terms of DNA repair is that the environment of a granuloma is hypoxic and hypoxia leads to DNA damage [54,55]. As identified by *in vitro*

**Table 2 Active phase of infection-affected genes**

All results are for MTB, unless otherwise described. +/–, Mutant increasingly susceptible or up-regulated gene/mutant presenting wild-type susceptibility or down-regulated gene; \*, at least a 1.8-fold change in expression level; †, for more data, see reference. SCID, severe combined immunodeficiency; vs., versus.

Locus	Gene	Mutant analysis				Expression analysis				
		Sensitive to				Broth culture		Murine		Human
		RNIs vs. wild-type <i>in vitro</i>	ROIs vs. wild-type <i>in vitro</i>	Alkylating agents vs. wild-type <i>in vitro</i>	Decreased survival rate in mice vs. wild-type	H <sub>2</sub> O <sub>2</sub> treated vs. untreated culture	Response to bovine surfactant or or its components [42]†	Macrophages	Whole-animal studies	Macrophages
Rv0054	<i>ssb</i>					+ [32]				
Rv0413	<i>mutT3</i>								+ (BALB/C and SCID mice) [47]	+ [108]
Rv0631c	<i>recC</i>								+ (only in SCID mice) [47]	+ [108]
Rv0861c	<i>ercc3</i>						–			
Rv0944,	<i>nei2</i>									
Rv0949	<i>uvrD1</i>	Ms + [26]	Ms + [26]							
Rv1020	<i>mfd</i>							+ [109]	+ (only in SCID mice) [47]	
Rv1160	<i>mutT2</i>							–		
Rv1210	<i>tagA</i>									
Rv1259	<i>udgB</i>	Ms + [110]								+ [43]
Rv1316c	<i>ogt</i>	+ [45]		– [45]		+ [32,44]*				
Rv1317c	<i>ada, alkA</i>	+ [45]		– [45]		+ [32,44]*		+ [44]*		+ [43]
Rv1329c	<i>dinG</i>							+ [109]		
Rv1420	<i>uvrC</i>									+ [46]
Rv1629	<i>polA</i>		Ms + [23]						+ (only in SCID mice) [47]	
Rv1633	<i>uvrB</i>	+ [25], Ms + [24,26]	– [25,115], Ms + [24,26]		+ [25]	+ [32]				
Rv1638	<i>uvrA</i>					+ [32]				+ [113]
Rv2090								+ [109], + [44]*		
Rv2191								+ [44]*		
Rv2592c	<i>ruvB</i>								+ (SCID mice and broth) [47]	
Rv2593c	<i>ruvA</i>									– [43]

**Table 2 (Contd).**

Locus	Gene	Mutant analysis				Expression analysis				
		Sensitive to				Broth culture		Murine		Human
		RNIs vs. wild-type <i>in vitro</i>	ROIs vs. wild type <i>in vitro</i>	Alkylating agents vs. wild-type <i>in vitro</i>	Decreased survival rate in mice vs. wild-type	H <sub>2</sub> O <sub>2</sub> treated vs. untreated culture	Response to bovine surfactant or its components [42]†	Macrophages	Whole-animal studies	Macrophages
Rv2697c	<i>dut</i>									+ [46]
Rv2719c						+ [44]*		+ [44]*		
Rv2736c	<i>recX</i>					+ [32,44]*		+ [44]*	+ (only in SCID mice) [47]	— [43]
Rv2737c	<i>recA</i>	Ms — [26]	Ms + [26]	BCG + [48]	BCG — [48]	+ [32,44]*				
Rv2836c	<i>dinF</i>							+ [109]	+ (BALB/C and SCID mice) [47]	
Rv2922c	<i>smc</i>								+ (only in SCID mice) [47]	
Rv2924c	<i>mutM</i> , <i>fpg</i>	Ms — [24]	Ms + [24,112]							
Rv2973c	<i>recG</i>							+ [44]*		
Rv2976c	<i>ung</i>	Ms + [24,110,111]	Ms + [24]							
Rv3014c	<i>ligA</i>								+ (only in BALB/C mice) [47]	
Rv3202c	<i>adnA</i>					+ [32,44]*		+ [44]*		
Rv3296	<i>lhr</i>					+ [44]*		+ [44]*		
Rv3394c	<i>dinX</i>					+ [32]				
Rv3585	<i>radA</i>					+ [32,44]*		+ [44]*		
Rv3589	<i>mutY</i>		Ms — [93]				+			
Rv3674c	<i>nth</i>						—			
Rv3715c	<i>recR</i>							— [44]*		

broth culture studies, several DNA repair genes of MTB are induced or repressed under hypoxic conditions [56–59] (for detailed information, see Supplementary Table S2 at <http://www.clinsci.org/cs/119/cs1190187add.htm>). However, even though there is a general trend for the down-regulation of DNA repair genes under such conditions, gene expression in different experiments differ greatly from one another. These results highlight that gene expression of mycobacteria might depend not only on their current conditions, but also on the way they achieved such a state, meaning that the results obtained by different methods might not be entirely comparable.

Another factor that influences the survival of mycobacteria in a granuloma is starvation. It has been shown that the response to such an environment is very quick; after 4 h of incubation in PBS several genes changed their expression, including *xseA*, which was down-regulated, and *mutT2* (Rv1160), which was up-regulated [60]. In long-term cultures (104 days) depleted of nutrients, several genes of MTB underwent changes in their expression profiles [61]. Some genes were maintained as being either up-regulated (*nfo*, *ada/alkA*, *ogt* and Rv2554c) or down-regulated (*recN*, Rv0269c, Rv2191 and *mpg*) for the extended period of time; however, most of the genes underwent several changes in their expression profiles (Supplementary Table S2). It was postulated that the change in expression profile at day 18 was a result of the stringent response as carbon sources were utilized, whereas at day 75 the population was progressing towards cell division, but changes in the expression profiles of DNA repair genes also occurred at other times [61].

Another factor that is probably encountered by mycobacteria within a granuloma is NO and related RNIs. Several lines of evidence suggest that NO is released in the lungs of people chronically infected with MTB (for more details, see [62]). Intriguingly, non-toxic concentrations of NO inhibit aerobic respiration and growth and, together with limited amounts of oxygen, prepare bacilli for survival in dormancy [63]. No DNA repair genes were up-regulated during exposure of mycobacteria to non-toxic concentrations of NO. Thus any influence of NO over DNA repair pathways in granulomas remains to be established.

*In vitro* studies of Ms deficient in DNA repair genes showed that *ung*- and *uvrB*-deficient mutants had increased sensitivity to hypoxia in broth culture [24]. Interestingly, comparison of mutant strains deficient in NHEJ and HR revealed that DSBs, at least those caused by ionizing radiation in the stationary phase, are preferentially repaired by NHEJ [22,28]. By contrast, a *recA*-deficient genotype of BCG did not affect growth in broth culture after oxygen depletion nor did it influence survival in BALB/C mice [48]. However, *recA*-deleted BCG harvested in oxygen-depleted medium had increased susceptibility to metronidazole, an antibiotic effective only with anaerobic bacteria, suggesting that

*recA* might have a role in maintaining genome stability at chronic stages of infection [48].

*In vivo* whole-genome studies bring additional important data about the influence of DNA repair genes during infection with MTB. Within artificial granulomas of mice (hollow fibre model), *uvrA* and *recF* became up-regulated [64]. *In vivo* studies in a rabbit model did not confirm these results as the only DNA repair gene that significantly changed its expression during latency, reactivation and immune reconstitution was Rv2191 [65]. Note that a hollow fibre applied by Karakousis et al. [64] allows the formation of an artificial granuloma carrying tubercle bacilli extracellularly, in contrast with a natural granuloma, where MTB is maintained within macrophages. Mice infected with a low dose of aerosolized MTB developed a persistent infection, which became chronic around days 45–60 of infection [66]. The results indicated that mycobacteria remained active, especially during the middle phase of chronic TB. At 60 days post-infection, *hupB* and *recO* were down-regulated and *ung* was up-regulated [66]. This expression profile was different from the profile encountered at the active stage of infection (day 15). Thus, while residing in granulomatous tissue, mycobacteria undergo several changes in their gene expression profiles (for more details see Table 3 and Supplementary Table S2).

Reactivation of infection begins when granulomas fail, and there are two main reasons for this phenomenon. One is the failure of the essential formation and maintenance of co-location signals from macrophages and T-cells, despite the presence of other responses that suggest the T-cells remain effective. The other reason relates to a decline in the host's immunity, which may result from the genetic susceptibility of the host or from environmental reasons, such as co-infection with HIV [51]. Little is known about the role of mycobacterial DNA repair genes in reactivation of infection; however, it seems that at least some of them are important for ensuring the survival of the bacteria. One such gene is *uvrB*, as an Ms strain deleted for this gene is compromised in its recovery from hypoxia in broth culture [24]. *In vivo* studies of dexamethasone-treated BALB/C mice, in which mice were undergoing immune suppression, revealed that expression of MTB genes *ogt*, *dinG* and *recA* were increased during reactivation [66]. *Ung* was up-regulated in the same experiment; however, it was also up-regulated in mice that were not undergoing immune suppression, and therefore presenting chronic TB.

Genome-wide expression analysis of mycobacteria from clinical human lung samples also confirmed the expression of tubercle bacilli DNA repair genes [67] (for more details, see Table 3 and Supplementary Table S2). Interestingly, even though the gene expression profiles of all isolates were similar, the profiles of up-regulated DNA repair genes were different in granuloma, pericavity and



**Table 3** MTB DNA repair genes up-regulated in granulomas *in vivo*

All results are for MTB. +, Indicates an up-regulated gene; \*, performed with artificial granulomas [MTB encapsulated in poly(vinylidene fluoride) hollow fibres implanted into the subcutaneous space]; †, results are on the basis of the supplementary material provided with the article, for more information see [67]. vs., versus.

Locus	Gene	Mouse [64]*	Mouse [66]		Rabbit [65]	Human [67]†		
			BALB/c mice at 45 vs. 15 days	BALB/c vs. broth at 60 days		Granuloma vs. broth	Pericavity/distant lung vs. granuloma	Pericavity/distant lung vs. broth
Rv0003	<i>recF</i>	+						
Rv0937c	<i>ku</i>					+		
Rv1638	<i>uvrA</i>	+						
Rv2090						+	+	+
Rv2191					+			
Rv2593c	<i>ruvA</i>						+	
Rv2836c	<i>dinF</i>						+	+
Rv2976c	<i>ung</i>			+				
Rv3201c	<i>adnB</i>		+					
Rv3394c	<i>dinX</i>					+		

distant lung tissue and only Rv2090 was up-regulated in all three of these isolates. The observed differences could be a result of differential physiology within the cells, but also because the pericavity and distant lung are exposed to air, leading to a heterogenous environment for the mycobacterial samples.

## MYCOBACTERIA, CHEMOTHERAPY AND EVOLUTION

One current important aspect of research into the pathogenesis of mycobacteria relates to the influence of added chemicals. An environment containing selective antibiotics promotes the occurrence of mutations in the pathogen's genome [68,69]. Consistent with these findings is the occurrence of drug-resistant strains of MTB, which have been divided into two main groups. MDR (multi-drug resistant) strains are resistant to two front line drugs, isoniazid and rifampicin, and these strains have been estimated to cover 5.3% of all new cases of TB around the world [1]. XDR (extensively drug-resistant) strains are resistant to isoniazid and rifampicin plus one of the fluoroquinolones and at least one second-line injectable drug (capreomycin, kanamycin or amikacin). Even though it is still to be established whether MTB possess plasmids [70,71] and if there is a functional conjugational apparatus in MTB [72,73], up to now all drug-resistance determinants are thought to be chromosomally encoded and to be a result of spontaneous nucleoid mutations [74] or gene inactivation by a mobile genetic element [75]. Mutations are acquired mainly by deletions, insertions or single nucleotide alterations or SNPs (single nucleotide polymorphisms). Acquired resistance towards anti-tubercular compounds has been

shown to occur in a single SNP in drug target genes, including *rpoB* [76–78], *katG* [76,77,79,80] and various others [81–86]. MTB harbours relatively little genetic diversity [5,87–89], but findings have shown that diversity between closely related strains may be greater than anticipated and it may be biologically significant [74,90]. Importantly, a larger amount of SNP diversity has been observed in XDR strains [91].

As in all organisms, DNA repair genes directly influence the rates and types of mutation that occur in mycobacteria. When they are functioning properly, these systems help maintain mutation rates at a low level, but in various circumstances they can increase the prevalence of specific types of mutations. First, DNA repair systems might not be efficient enough to repair large amounts of DNA damage, which can therefore lead to mutations. Secondly, some DNA damage repair systems, such as NHEJ [28] or translesion synthesis by DnaE2 [32,92], are error-prone and the repair pathway can lead directly to mutated sequences. Finally, mutations to DNA damage repair proteins can lead to an increase in the mutation rate, as has been observed upon deletion of *ung*, *uvrB*, *uvrD*, *fpg* and *udgB* in *Ms* and *ada/alkA* together with *ogt* in MTB (Table 4 and Supplementary Table S3 at <http://www.clinsci.org/cs/119/cs1190187add.htm>). Furthermore, *mutY* [93] and *mutT1* [94] mutations have been observed in drug-resistant phenotypes. Analysis of isoniazid-resistant strains has suggested that the SNPs responsible for resistance had arisen independently [82].

Certain genotypes of mycobacteria are particularly prone to acquiring mutations that result in drug resistance. SNPs seem to be a driving force for two common drug-resistant lineages of MTB, W-Beijing and Haarlem. Both of these strains are extremely fit for

**Table 4** Response of DNA repair genes to chemotherapy by first- and second-line drugs and the influence on mutation acquisition

All results are for MTB, unless otherwise stated. +/—, Indicates an up-regulated gene or an increased mutation rate in a deficient mutant/down-regulated gene; \*, performed with artificial granulomas [MTB encapsulated in poly(vinylidene fluoride) hollow fibres, implanted into the subcutaneous space]; †, at least a 2-fold change in expression level compared with untreated cultures.

Locus	Gene	Isoniazid [103]	Isoniazid [106]				Capreomicine [104]	Rifampicin [31]†	Streptomycin [31]†	Amikacin [31]†	Levofloxacin [31]†	Ofloxacin [31]†	Increased mutation rate if absent
			Exponential phase growth	Dormancy*	Nutrient depletion	Progressive hypoxia							
Rv0003	<i>recF</i>			—		—							
Rv0054	<i>ssb</i>						+						
Rv0269c		+											
Rv0427c	<i>xthA</i>											+	
Rv0630c	<i>recB</i>	—							—				
Rv0670	<i>nfo, end</i>	+			+								
Rv0938	<i>ligD</i>	+											
Rv0944,	<i>nei2</i>												
Rv0949	<i>uvrD1</i>												Ms + [26]
Rv1021	<i>mazG</i>	+				—							
Rv1210	<i>tagA</i>	—										+	
Rv1259	<i>udgB</i>												Ms + [110,114]
Rv1316c	<i>ogt</i>											+	+ [45]
Rv1317c	<i>ada, alkA</i>										+	+	+ [45]
Rv1420	<i>uvrC</i>	—											
Rv1537	<i>dinX</i>				+			+					
Rv1629	<i>polA</i>	+											
Rv1633	<i>uvrB</i>											+	Ms + [24,26]
Rv1638	<i>uvrA</i>										+		
Rv2191										+			
Rv2592c	<i>ruvB</i>		—										
Rv2594c	<i>ruvC</i>		—										
Rv2697c	<i>dut</i>												

**Table 4 (Contd.)**

Locus	Gene	Isoniazid [103]	Isoniazid [106]				Capreomicine [104]	Rifampicin [31]†	Streptomycin [31]†	Amikacin [31]†	Levofloxacin [31]†	Ofloxacin [31]†	Increased mutation rate if absent
			Exponential phase growth	Dormancy*	Nutrient depletion	Progressive hypoxia							
Rv2719c												+	
Rv2720	<i>lexA</i>	+											
Rv2737c	<i>recA</i>											+	
Rv2924c	<i>mutM, fpg</i>	—											Ms + [24,112]
Rv2973c	<i>recG</i>	—											
Rv2976c	<i>Ung</i>												Ms + [24,110,111]
Rv2985	<i>mutT1</i>					+							
Rv2986c	<i>hupB</i>		—										
Rv3198c	<i>uvrD2</i>												
Rv3201c	<i>adnB</i>	+											
Rv3202c	<i>adnA</i>	+											
Rv3296	<i>lhr</i>											+	
Rv3297	<i>nei</i>												
Rv3395c	<i>dinF</i>										+		
Rv3585	<i>radA</i>											+	
Rv3674c	<i>nth</i>				—								
Rv3715c	<i>recR</i>		—				+			+			
Rv3731	<i>ligC</i>	—											
Rv3908	<i>mutT4</i>												

survival as they account for the majority of strains identified in many countries [95–99] and both account for a vast number of MDR and XDR strains [98–100]. It has been discovered that many of these drug-resistant strains carry mutations in DNA repair genes, resulting in their mutator phenotype. *ada/alkA*, *ogt* [94,101] and *ung* [101] mutations have been discovered in Haarlem lineages, whereas *mutT4*, *mutT2* and *ogt* have been discovered in W-Beijing lineages [100,102]. The same mutations are also observed in non-MDR strains, but this does not negate the point that their mutator phenotype may increase the possibility of them acquiring mutations against anti-tubercular drugs.

Many anti-tubercular compounds influence the expression of DNA repair genes in mycobacteria [29,31,103–106] (Table 4 and Supplementary Table S3), including first- and second-line drugs that are currently used in therapy (Table 4). Although no DNA repair genes were induced in response to the first-line drugs pyrazinamide or ethambutol, under treatment with ofloxacin, an inhibitor of DNA gyrase, ten of them were up-regulated at least 2-fold [31]. It is, thus, a tempting idea that the use of currently applied antibiotics combined with drugs that target the up-regulated DNA repair genes might increase the effectiveness of any chemotherapy. In fact, a *recA*-deficient mutant of BCG had increased susceptibility to metronidazole *in vitro* [48], and an *ogt* mutant of BCG had increased susceptibility to isoniazid [107]. However, it would be important to test such strategies carefully to ensure that the reduction in the activity of DNA repair proteins does not result in an increased mutation rate that could contribute to the generation of drug-resistant strains.

## CONCLUSIONS

DNA repair pathways in mycobacteria are crucial for their survival at different stages of infection. As infection progresses, different sets of genes play an important role in maintaining the stability of the mycobacterial genome. Even though results obtained using different methods are sometimes contradictory, an improved understanding of the role of DNA repair in the pathogenesis of mycobacteria might reveal good candidates for effective treatment against tuberculosis. There is much data regarding the expression of each DNA repair gene in MTB; however, there is still a lot to discover and more studies, especially performed *in vivo* with MTB, are needed.

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