Nucleotide Excision Repair Pathway in Mycobacteria

16

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Abstract

Nucleotide excision repair (henceforth abbreviated as NER) plays a pivotal role in all organisms to protect their genetic material against radiations, toxic chemicals, and normal by-products of cellular metabolism. In humans, defects in excision repair causes inherited diseases, and NER-related human diseases are associated with cancer and aging. Much of our current understanding of NER has emerged from experimental evidence in model systems including *Escherichia coli*, yeast, and mammalian cells. Considering the importance of NER in the maintenance of genome integrity, it is surprising that only a few studies have investigated NER in mycobacteria. Here we provide a brief overview of the mechanism of the DNA repair in mycobacteria. A detailed understanding of structure-function relationship of DNA repair proteins in tubercle bacillus could facilitate the identification and development of novel therapeutic targets for tuberculosis therapy.

Keywords

DNA damage · DNA helicase · *Mycobacterium tuberculosis* UvrA · UvrB · UvrC · UvrD · Nucleotide excision repair · SOS response

Abbreviations

ATP	adenosine triphosphate
BCG	Bacillus Calmette–Guerin
BER	base excision repair;
CFU	colony-forming units

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DSBs	double-strand breaks
dsDNA	double-stranded DNA
EcRecA	*Escherichia coli* RecA
EcUvrA	*Escherichia coli* UvrA
EcUvrB	*Escherichia coli* UvrB
HR	homologous recombination
iNOS	inducible nitric oxide synthase
MDR	multidrug resistant
MMR	mismatch repair
MtRecA	*Mycobacterium tuberculosis* RecA
MtUvrB	*Mycobacterium tuberculosis* UvrB
NER	nucleotide excision repair
NHEJ	non-homologous end joining
RNI	reactive nitrogen intermediates
ROI	reactive oxygen intermediates
ssDNA	single-stranded DNA
TCR	transcription-coupled repair
UV-light	ultraviolet-light
XDR	extensively drug resistant

16.1 Overview of DNA Damage Response Mechanisms

Numerous studies have established that the genomic DNA undergoes damage inflicted by radiation, chemicals, environmental agents, and internal metabolic (endogenous) processes (Hoeijmakers 2001; Lindahl and Wood 1999). These produce nucleotide modifications such as bulky adducts, single- and double-strand breaks, inter- or intra-strand cross-links, DNA-protein cross-links, apurinic or apyrimidinic base-free sites, misincorporation of nucleotide or ribonucleotide by DNA polymerases, and DNA replication slippage (Vaisman et al. 2013; Dexheimer 2012; Lindahl and Wood 1999; Van Houten and Sancar 1987). If these lesions remain unrepaired, they lead to genomic instability and various diseases like cancer, neurological disorders, immunodeficiency, premature aging, and Fanconi anaemia in humans (Iyama and Wilson 2013). Therefore, maintenance of genome stability under normal and stressed conditions becomes an important task to the cellular repair machinery.

To circumvent the harmful or deleterious effects of myriad types of DNA damage, cells have evolved specialized types of DNA repair systems. These can be classified based on the protein components and mechanism(s) involved as well as different types of DNA lesions being processed. The different DNA damage repair pathways include direct reversal of damaged base, base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), and single- and double-strand breaks (DSBs) repair (Fig. 16.1). The repair of both single- and

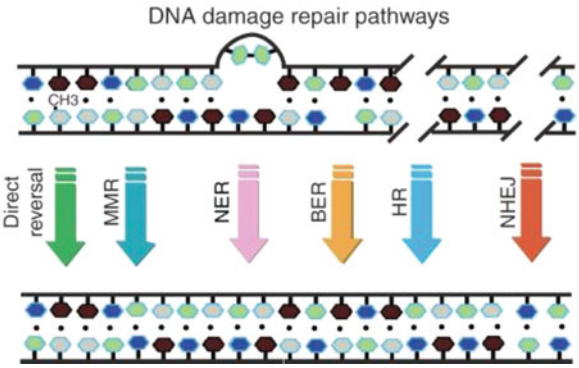

Fig. 16.1 Overview of DNA damage and repair mechanisms. The figure illustrates common types of DNA lesions and the specific mechanism to repair each type of lesion (this Figure is cited from Hakem (2008). (Reproduced with the permission of EMBO J)

double-strand breaks involves homologous recombination (HR) and non-homologous DNA end-joining (NHEJ) pathways (Kuzminov 2001; Cannan and Pederson 2016; Friedberg 2016; Yi and He 2013; Hakem 2008). The repair of damaged bases involves three mechanistically distinct processes: (1) photolyase catalysed reversal of UV light-induced photolesions; (2) O^6-alkylguanine-DNA alkyltransferases (AGTs) catalysed reversal of various O-alkylated DNA damage; and (3) the AlkB family dioxygenases mediated reversal of N-alkylated base adducts (Yi and He 2013). The photolyase enzyme reactivates DNA by direct repair of T<>T lesions, and BER is used to repair single-stranded breaks and to correct the damaged bases resulting from spontaneous DNA deamination, hydroxylation of bases, or exposure of DNA to alkylating agents. On the other hand, MMR recognizes and repairs erroneous insertions, deletions, and misincorporation of bases arising from DNA replication, and base deamination. This system recognizes the presence of hemimethylated dGATC sequences that provides molecular basis to distinguish between the newly synthesized daughter strand and parental DNA strand (Radman and Wagner 1986). This hemi-methylated dGATC site 5′ or 3′ to the mismatch then cleaved by the concerted action of MutS, MutL, and MutH in an ATP-dependent manner (Acharya et al. 2003). ExoI or ExoX (3′ → 5′ exonuclease), or ExoVII or RecJ (5′ → 3′ exonuclease) excise the cleaved strand up to and slightly past the mismatch. The resulting single-stranded gap is repaired by DNA synthesis using intact complimentary strand as a template and ligated by DNA polymerase III or DNA ligase (Li 2008).

The double-stranded breaks are repaired via either HR or NHEJ, but what determines the pathway preference remains elusive (Lieber 2010). In eubacteria, HR comprises of four stages: (1) when DSBs occur, a process called DNA end resection is activated to generate 3′ single-stranded overhangs; (2) RecA polymerizes onto 3′-single-stranded DNA (ssDNA) to form a nucleoprotein filament; (3) RecA nucleoprotein filament initiates search for homology, pairing, and strand exchange; and (4) finally, the extension of heteroduplex DNA by RuvAB followed by resolution of the Holliday junction by RuvC produces crossover and non-crossover products (Cox 2007; Kowalczykowski et al. 1994). The bacterial cells also depend on NHEJ during pre-replicative stage when only single copy of the

genome is available. Genetic and biochemical evidences have also shown that mycobacteria possess NHEJ pathway that depends on DNA ligase (LigD) and Ku protein (Matthews and Simmons 2014; Shuman and Glickman 2007). Although the biochemical mechanisms of all these pathways have been extensively studied in considerable depth in bacteria, how these repair events are modulated within the nucleoid is not fully understood.

16.2 DNA Damage and SOS Response

The induction of the SOS response in bacteria is a global response to DNA damage in which cell division is arrested and DNA repair is activated by the expression of DNA damage repair genes (Adikesavan et al. 2011; Schlacher and Goodman 2007; Radman 1975). In *Escherichia coli*, the SOS response involves two key proteins: LexA (a repressor) and RecA (an inducer) as shown in Fig. 16.2 (Radman 1975). Under normal growth conditions, LexA dimer binds to a 20-base pair consensus palindromic DNA sequence (also known as the SOS box) and represses the transcription of a regulon (comprising of more than 50 genes, including *lexA* and *recA*)

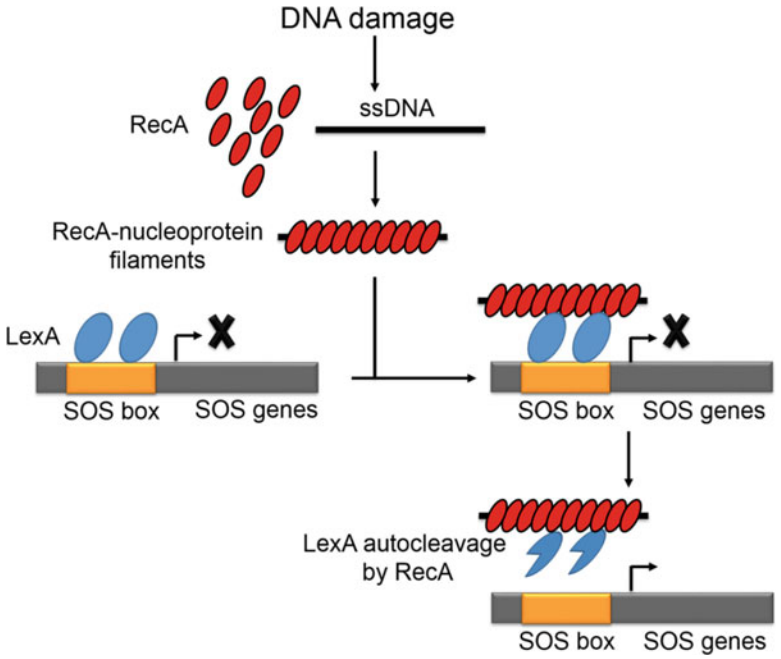

Fig. 16.2 DNA damage and SOS response. Initially the LexA repressor binds to SOS box, present upstream of SOS genes. Under DNA-damaging conditions, the single-stranded DNA (ssDNA) that originates from double-strand breaks (DSBs) repair pathway binds RecA proteins leading to the formation of RecA-nucleoprotein filaments. These filaments induce the autocatalytic cleavage of LexA allowing derepression of SOS genes. (Figure adapted from Butala et al. 2009)

(Adikesavan et al. 2011). Upon DNA damage, RecA forms a nucleoprotein filament on ssDNA (Cox 2007; Kowalczykowski et al. 1994). The RecA nucleoprotein filament facilitates the self-cleavage of LexA, leading to the derepression of SOS regulon genes (Kowalczykowski et al. 1994). The *uvr* genes are among the first to be induced, followed by *lexA* and *recA* genes, while genes encoding the low fidelity, error-prone repair DNA polymerases Pol II (*polB*), Pol IV (*dinB*), and Pol V (*umuC* and *umuD*) are induced under extensive and persistent DNA-damaging conditions (Butala et al. 2009; Smith and Sharma 1987; Smith et al. 1987). These DNA polymerases allow DNA replication over the DNA lesions (translesion DNA synthesis) that block the primary replicative DNA polymerase Pol III and leads to mutations in the newly replicated and repaired regions. The *uvrA*, *uvrB*, and *ydjQ* (*cho*) genes are SOS-induced genes, whereas *uvrC* gene is not regulated by SOS response (Janion 2001, 2008; Simmons et al. 2008; Bohr 1991; Calsou and Salles 1985; Youngs et al. 1974). Although the mechanism of UvrABC mediated repair of UV-induced photoproducts have been studied extensively under in vitro conditions, how this system is organized in vivo is unclear. Some estimates indicate that at least 17 protein components are recruited to the inner membrane of *E. coli* after UV irradiation. The colocalization of UV-induced 6–4 photoproducts and NER proteins to the inner membrane following UV-irradiation suggests that a part of the repair process may associate with the inner membrane (Lin et al. 1997).

The *Mycobacterium tuberculosis recA* gene encodes an 85 kDa precursor, which is processed to generate a 38 kDa functionally active RecA and 47 kDa intein (Davis et al. 1991; Kumar et al. 1996). The X-ray structure of *M. tuberculosis* RecA (MtRecA) is very similar to that of *E. coli* RecA (EcRecA) (Datta et al. 2000). The transcriptional regulation of *M. tuberculosis recA* differs from that of *E. coli recA: M. tuberculosis recA* is regulated by two promoters (Movahedzadeh et al. 1997b). One of the promoters is regulated by LexA repressor, while the second promoter is DNA damage-inducible, but independently of both LexA and RecA (Davis et al. 2002b). The *M. tuberculosis* LexA-binding site (SOS box) is similar to that of *Bacillus subtilis* (Cheo box) (Movahedzadeh et al. 1997a). Further investigations revealed the existence of several LexA-regulated genes in *M. tuberculosis* (Davis et al. 2002a). The apparent lack of correlation between the expression of DNA damage-inducible genes and the LexA repressor in *M. tuberculosis* supports an alternative mechanism of LexA-independent DNA damage induction (Brooks et al. 2001). Despite this, *M. tuberculosis* genome possesses the canonical core of the SOS system, except *polB* and *umuD* (Cole 1998; Mizrahi and Andersen 1998). The global gene expression following DNA damage in both wild-type and *recA*$^{-}$ strain of *M. tuberculosis* revealed that most of the genes induced by DNA damage do not have any known roles in DNA repair. However, another set of the inducible genes with a known or predicted function in DNA repair was found to be independent of *recA* (Rand et al. 2003). This study also revealed yet another group of genes, *lexA* itself, *ruvA*, *ruvB,* and *dnaE2* whose expression was strictly dependent on *recA* for induction and group of genes whose expression appear to be regulated by both *recA*-dependent and *recA*-independent mechanisms, e.g. *recA* itself, *radA*, *ruvC*, and *ssb*.

16.3 Discovery of Nucleotide Excision Repair Genes

Briefly, the survival of *E. coli* K-12 cells following UV radiation led to the discovery of NER genes (Boyce and Howard-Flanders 1964; Setlow and Carrier 1964; Howard-Flanders et al. 1962a). In early studies, thymine dimers were found to be the main photoproducts after UV irradiation (Setlow and Setlow 1962, 1963; Setlow et al. 1963). The identification of repair-deficient mutants was based on contra-selection of irradiated T1 bacteriophage particles (Howard-Flanders and Theriot 1962). These studies identified three loci in *E. coli* K-12 that control the excision of pyrimidine dimers and these genes were designated as *uvrA*, *uvrB*, and *uvrC* (Setlow and Carrier 1964; Howard-Flanders et al. 1962a, b, 1966).

16.4 Overview of Nucleotide Excision Repair Pathway

Studies over the past four decades have identified distinct DNA repair pathways and molecular mechanisms underlying the myriad types of biochemical reactions of various repair systems. Among various forms of genomic DNA damage, "bulky" DNA lesions that cause helical distortion in the double helix can lead to replication fork arrest, and mutations (Reardon and Sancar 2005). The bulky chemical adducts and interstrand cross-links are the outcomes of UV irradiation and various other mutagens (Kisker et al. 2013; Truglio et al. 2006; Jeggo et al. 2015). In all three domains of life, various proteins that belong to distinct DNA repair pathways recognize these DNA alterations and then either repair them by direct reversal (photoreactivation system) or target them for removal by a series of DNA repair systems (Reardon and Sancar 2005; Kisker et al. 2013; Truglio et al. 2006; Jeggo et al. 2015). One of the most studied and best understood DNA repair machinery in eubacteria and archaebacteria is the NER pathway (Jeggo et al. 2015; Kisker et al. 2013; Truglio et al. 2006; Reardon and Sancar 2005; Ogrunc et al. 1998; Grogan 2000). In these two domains, the NER pathway is dedicated to the removal of a variety of chemically and structurally diverse DNA lesions, including UV-induced photoproducts (cyclobutane dimers, 6–4 photoproducts, and thymine glycol), bulky adducts, apurinic or apyrimidinic (AP) sites, and cross-links, albeit with variable efficiencies (Goosen 2010; Truglio et al. 2006; Reardon and Sancar 2005; Alekseyev et al. 2004; Selby and Sancar 1991; Snowden et al. 1990; Lin and Sancar 1989; Van Houten et al. 1988; Van Houten and Sancar 1987; Thomas et al. 1986; Sancar et al. 1985; Seeberg et al. 1983). In addition to the structural distortions in the DNA, UvrABC can also repair cross-links between protein and DNA as well as the damages caused by reactive oxygen and nitrogen species (Salem et al. 2009; Imoto et al. 2008; Minko et al. 2002, 2005; Nakano et al. 2005).

In all three domains of life, NER comprises of discrete steps: DNA scanning, damage detection, damage verification, incision or removal of damage, DNA synthesis, and ligation as depicted in Fig. 16.3 (Hu et al. 2017; Kisker et al. 2013;

Fig. 16.3 (continued) is required for the removal of incised lesion containing small oligonucleotide. Repair is then completed by the action of DNA polymerase I and DNA ligase which synthesizes the new strand using intact complementary strand as a template and ligates the nick, respectively. (Figure adapted from Kisker et al. 2013)

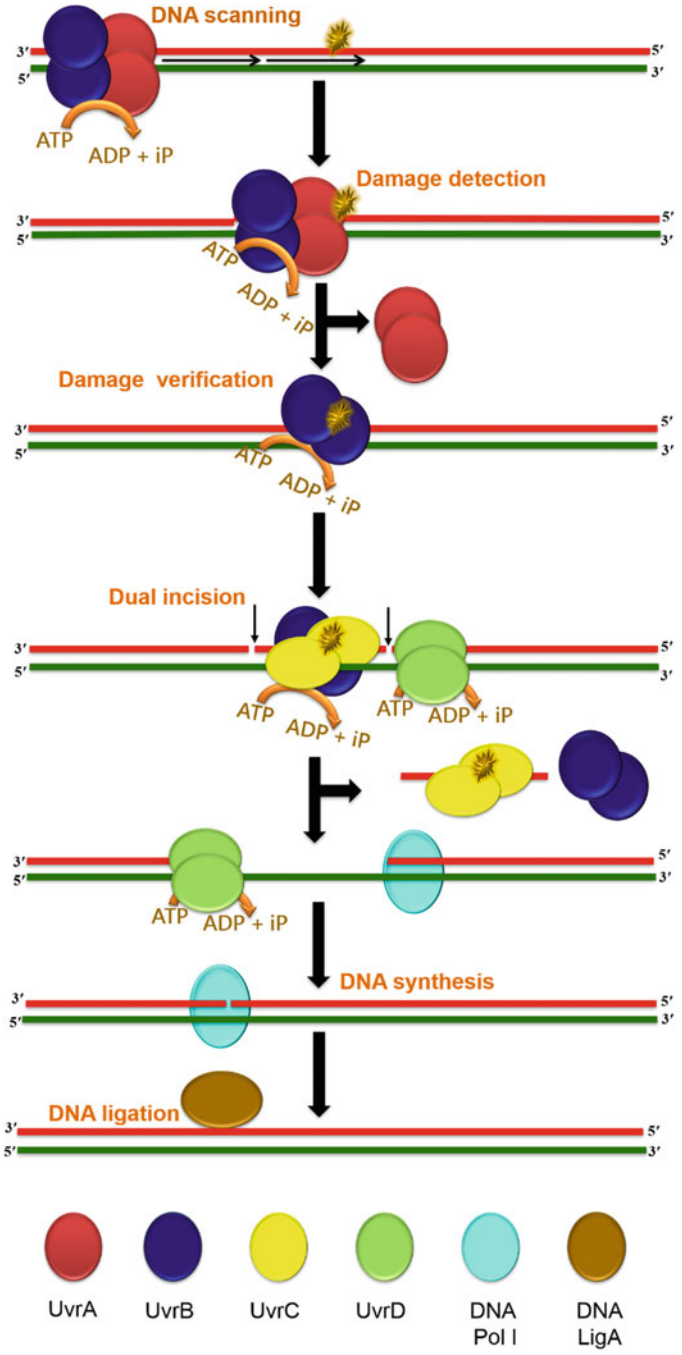

Fig. 16.3 Schematic representation of the prokaryotic NER pathways. Genome is scanned by the heterotetrameric $UvrA_2$–$UvrB_2$ complex in search for damaged nucleotides in an ATP-dependent manner. After the damage detection by UvrA component, pathway proceeds with damage verification by UvrB followed by 3′ and 5′ incisions catalysed through UvrC. The helicase activity of UvrD

Truglio et al. 2006; Ogrunc et al. 1998;). In prokaryotes, NER can be initiated by two major pathways often referred to as global genome repair (GGR) and transcription-coupled repair (TCR). GGR can take place at any region of the genome and is generally initiated by the direct binding of UvrAB complex to the DNA under DNA-damaging conditions or during the SOS response (Simmons et al. 2008; Janion 2001, 2008; Crowley et al. 2006; Hanna et al. 2001). The transcription coupled NER is initiated by stalled RNA polymerase on the template strand with the help of TCR specific factor, Mfd (Pani and Nudler 2017; Van Houten and Kad 2014). The TCR not only leads to repair of lesions on the DNA but also accomplishes efficient and correct transfer of information from genes to RNA. Although the first step of initiating NER is different in GGR and TCR, both the processes require the same molecular protein complexes to complete the excision repair (Van Houten and Kad 2014). Specifically, UvrAB complex bound to ATP scans the genome for lesions. Once a lesion is detected by UvrA, the lesion containing strand is transferred to the UvrB for damage verification and UvrA is released from the process (Pakotiprapha et al. 2012; Jaciuk et al. 2011; Kad et al. 2010; Malta et al. 2007; Truglio et al. 2006). The UvrB subunit binds specifically to the lesion containing DNA strand by inserting its beta-hairpin domain between the two strands of the DNA for verification of the lesion. UvrC binds to this tight UvrB-DNA preincision complex and mediates the incisions on both 3′ and 5′ sides of the lesion on the single-stranded DNA (Pu et al. 1989). Many bacterial species such as *E. coli*, *Mycobacterium tuberculosis, Salmonella typhimurium, Salmonella enteritidis, Klebsiella pneumoniae, and Clostridium acetobutylicum* harbour another protein called YdjQ (renamed as Cho) which incises only at the 3′ side of the lesion. It was found that Cho cleaves the poor DNA substrates of UvrC very efficiently and thus further increases the substrate range of NER (Moolenaar et al. 2002). Subsequently, UvrD (DNA helicase II) removes the excised lesion containing the oligonucleotide by its helicase action and DNA polymerase I fills the gap using intact complimentary strand as a template (Brosh 2014; Lee and Yang 2006; Runyon et al. 1990; Caron et al. 1985). Finally, DNA ligase seals the nick; thus genome integrity is restored (Paul-Konietzko et al. 2015; Orren et al. 1992; Van Houten et al. 1988; Husain et al. 1985).

Much of the available knowledge regarding NER has come through investigations in model organisms such as *E. coli*, yeast, and cultured mammalian cells. A detailed discussion on the mechanistic aspects of NER is beyond the scope of this review; however, the reader is referred to excellent reviews that deal with the mechanistic aspects of NER (Hu et al. 2017; Pani and Nudler 2017; Kisker et al. 2013; Van Houten et al. 2005). While the NER pathway has been studied

extensively over the years, the comparison between *E. coli* and mycobacteria is important for identifying possible genetic and mechanistic differences that might contribute to the observed differences. This review focuses on the function of NER in mycobacteria and on the role of this pathway in the cellular response to UV-induced DNA damage.

16.5 DNA Repair in *Mycobacterium tuberculosis*

The macrophages mount an effective host defence by recognizing, engulfing, and killing *M. tuberculosis* (Levitte et al. 2016). The recruitment of macrophages is through Toll-like receptor (TLR)-mediated signalling that is triggered by pathogen-activated molecular patterns (PAMPs) present on bacterial surface (Fig. 16.4). The infected macrophages migrate across the lung epithelium and then transport the

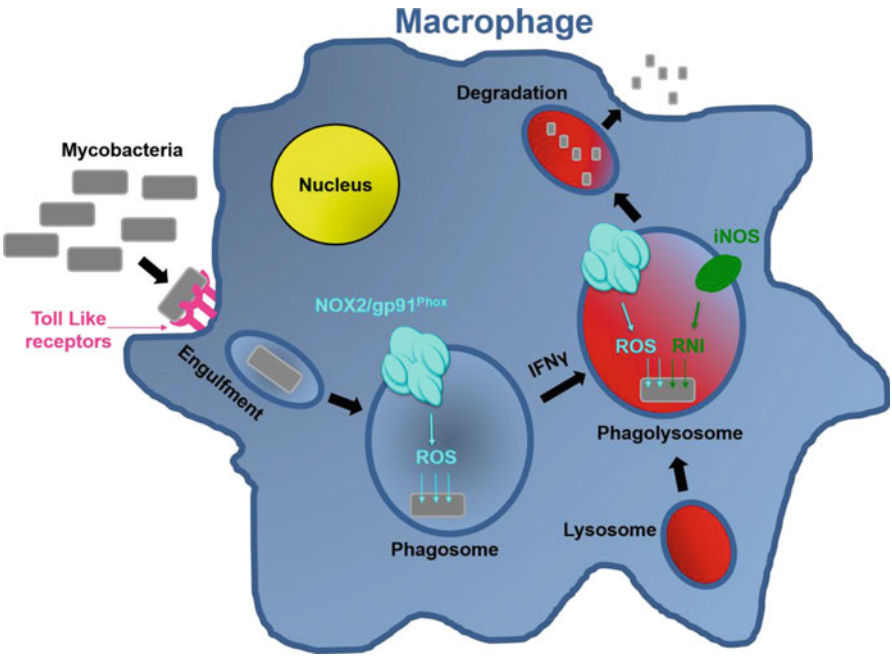

Fig. 16.4 Simplified diagram of the phagocytosis and degradation of a mycobacterial cell. Phagocytosis is triggered by the binding of Toll-like receptors to the PAMPs on the cell surface of mycobacteria. After the particle is taken up by the phagocyte, mycobacterial DNA is exposed to the deleterious effect of ROI generated by the action of phagocyte oxidase ($NOX2/gp91^{Phox}$). Upon immunological activation with IFNγ, the phagosome matures and fuses with lysosomes leading to the production of RNI from iNOS (inducible nitric oxide synthase 2) and ROI from NOX2. These species react with a wide range of molecules, including nucleic acids, proteins, lipids, and carbohydrates. If the damaged DNA of *M. tuberculosis* left unrepaired, the mycobacterial cells inside the macrophages will undergo apoptosis inside phagolysosomes, and subsequently the cell debris will be secreted out by the phagocyte. (Figure adapted from Ehrt and Schnappinger 2009)

bacteria to other tissues (Cambier et al. 2014a). Subsequently, new macrophage recruitment takes place and the original infected macrophages form granuloma, an aggregation of differentiated macrophages, and other immune cells. In these granulomas the infection expands by spreading bacteria to newly arrived macrophages (Cambier et al. 2014b). The experimental evidences suggest a link between the association of Toll-like receptor signalling, reactive oxygen intermediates (ROI), and reactive nitrogen intermediates (RNI) (Marcato et al. 2008; Ryan et al. 2004). Such signalling cascades lead to the activation of macrophages as an innate immune response to kill *M. tuberculosis*. Studies suggest that both ROI and RNI can permeabilize the cell wall/membrane of the pathogen (Fig. 16.4) (Schlosser-Silverman et al. 2000; Fang 1997). Most common damages that occur in DNA are the base modifications, generation of abasic sites, and strand breaks in response to RNI and ROI (Wink et al. 1991). Specifically, ROS produces single pyrimidine and purine base lesions, intrastrand cross-links, purine 5′,8-cyclonucleosides, DNA-protein adducts, and interstrand cross-links (Cadet and Wagner 2013). Therefore, inability to rectify these damages in the DNA inside the macrophages can compromise its survival and ability to cause pathogenesis. Several studies suggest that *M. tuberculosis* is well equipped to counteract the harmful effects of ROS, RNI, and low pH (<3.5) generated by the host immune system (Ehrt and Schnappinger 2009). But how the genome integrity of *M. tuberculosis* is maintained under these conditions continues to be an important area of research. To this end, genome-based studies in *M. tuberculosis* showed the involvement of various DNA repair proteins and nucleoid-associated proteins (NAPs) to circumvent such DNA damaging conditions (Philipp et al. 1998a, b; Bergh and Cole 1998; Brosch et al. 1998).

M. tuberculosis harbours all the vital genes of NER, base excision repair (BER), HR, NHEJ, SOS repair, and mutagenesis, as deduced from bioinformatics and genome-based studies, but the function of these proteins and enzymes is not fully understood. Moreover, studies on DNA repair components is important considering that *M. tuberculosis* lacks functional mismatch repair pathway (MMR) (Cole 1998). In contrast to *Helicobacter pylori*, in which a correlation exists between the absence of MMR and high level of genetic diversity, *M. tuberculosis* genomes are very stable (Kang and Blaser 2006). This observation suggests that other DNA repair mechanisms could be very efficient in this pathogen. Recently, NucS-dependent DNA repair system that mimics the MutS/MutL-based MMR has been identified in *Mycobacterium smegmatis* (Castaneda-Garcia, et al. 2017). Thus, characterization of *M. tuberculosis* DNA repair pathways in vivo and in vitro may contribute to the understanding of the generation of clonal populations of *M. tuberculosis* (Sreevatsan et al. 1997).

The adaptive nature of *M. tuberculosis* is significant in the clinical context because of its ability to supress central metabolism, halt DNA replication, and enter into a stage of dormancy rendering itself extremely resistant to host defence and drug treatments (Gengenbacher and Kaufmann 2012). Thus, the physiological changes that take place during the shift from dormancy to reactivation of the tubercle bacillus should be investigated in detail (Gopinath et al. 2015; Ehrt et al. 2015;

Gorna et al. 2010). To this end, Affymetrix GeneChip System Microarray (based on a specific oligonucleotide array format) revealed that almost half of the DNA repair genes are continuously expressed during log phase of growth, indicating that the bacteria continuously counteract the constant exposure to DNA-damaging conditions (Fu and Fu-Liu 2007). Additionally, transposon site hybridization (TraSH) technique identified the genes, Rv2554c, *dut*, *ligA*, *polA*, and *adnB* and those encoding the NER-related proteins UvrD1, UvrD2, and UvrC, which are essential for optimal growth by *M. tuberculosis* (Sassetti et al. 2003). The *uvrD2* and *ligA* genes were also found to be essential for the survival of *M. smegmatis* (Sinha et al. 2008; Korycka-Machala et al. 2006). In another study, every nonessential gene of *M. tuberculosis* was disrupted and their effect on the growth rate and virulence was determined in mice models. This study also revealed a set of DNA repair proteins required for survival in mice, apart from a variety of proteins from different pathways. These include three BER proteins (Ung, Nfo/End, and XthA), MazG, and RecN (Sassetti and Rubin 2003). The proteome profiling of *M. tuberculosis* also indicated the role of DNA repair genes during dormancy and reactivation (Gopinath et al. 2015). Altogether these studies suggest that most of the *M. tuberculosis* DNA repair genes are nonessential for survival in the broth, disruption of these genes lead to attenuation of virulence in mice. A possible link between survival and spatial-temporal regulation of DNA repair proteins has been established by the isolation of *M. tuberculosis* W-Beijing strains. These strains belong to a drug resistance family which could be divided into several branches based on unique alterations in three putative antimutator genes: *mutT4, mutT2*, and *ogt* (Dos Vultos et al. 2008; Nouvel et al. 2007; Ebrahimi-Rad et al. 2003).

M. tuberculosis infection is transmitted by aerosol droplets containing bacteria, coughed out by individuals with active disease, into the lower lungs of a new host. Various studies show that *M. tuberculosis* viability in the aerosols decline to 55%, 10%, and almost zero after 5, 30, and 60 min, respectively (Lever et al. 2000; Loudon et al. 1969). Studies based on artificially generated droplets carrying BCG pointed out that UV radiation and desiccation sterilize mycobacterial contaminated air (Peccia and Hernandez 2004; Ko et al. 2002; Riley et al. 1976). Nevertheless, from these studies it is evident that the viability of mycobacteria drastically reduces in the aerosols. It is known that intracellular dehydration (desiccation) leads to DSBs in DNA, whereas UV exposure leads to the generation of cyclobutane pyrimidine dimers and pyrimidine (6–4) pyrimidine photoproducts, in which two adjacent pyrimidines are covalently linked to each other. In mycobacteria, the HR and NHEJ pathways are involved in the repair of DSBs. Interestingly, *M. smegmatis* mutant strains lacking RecA and Ku and/or LigD showed that neither of these proteins are essential for its growth in vitro (Korycka-Machala et al. 2006). Despite this, both processes are required for survival under desiccation in the broth culture (Pitcher et al. 2007). Similar studies were also performed by generating mutants that influence NER in *M. smegmatis* and *M. tuberculosis*. Mutations in *polA*, *uvrB*, and *uvrD1* of *M. smegmatis* and *uvrB* in *M. tuberculosis* lead to increased susceptibility to UV radiation in vitro (Guthlein et al. 2009; Darwin and Nathan 2005; Gordhan et al. 1996). The deletion of both *uvrB* and *uvrD1* in *M. smegmatis* showed additive

effect on survival in response to UV radiation indicating that one of the proteins is involved in another DNA repair pathway. The microarray analysis of *M. tuberculosis* genes induced under UV treatment in broth cultures indicated that the levels of expression of HR and NER gene products increase significantly. These proteins include UvrA, UvrB, UvrD2, PolA, RecA, RuvC, RuvA, LexA, RecX, and AdnA (Boshoff et al. 2003, 2004). These studies also highlighted a role for several other proteins like Ogt, Ada/AlkA, DinF, Lhr, DinX, and DnaE2 in DNA repair. Besides this, neither the relationship between HR and NER proteins nor the effect of double mutations in NER and HR proteins is completely understood in mycobacteria.

16.6 DNA Repair Pathways Are Active During Macrophage Infection

The genome of *M. tuberculosis* is exposed to DNA-damaging conditions inside the granulomas (Adams et al. 1997; Moller et al. 2001). Mice with a non-functional allele of gp91phox subunit of phagocyte oxidase cytochrome b and iNOS KO mice lacking an inducible nitric oxide synthase (iNOS) gene fail to produce RNS. Both these strains of mice experience exaggerated growth of mycobacterial infection (Adams et al. 1997; MacMicking et al. 1997; Chan et al. 1995). The microarray analysis revealed that the expression levels of genes involved in NER and BER are upregulated in the activated macrophages. Apart from these, *M. tuberculosis ada/alkA* transcript levels increase significantly in human or murine macrophages as well as in response to H_2O_2 in broth culture (Fontan et al. 2008; Schnappinger et al. 2003). Moreover, deletion of *ada/alkA* gene in *M. tuberculosis* leads to increased susceptibility to RNIs, but survival was found to be unaffected (Durbach et al. 2003). Similarly, deletion of *uvrB* gene in *M. smegmatis and M. tuberculosis* also lead to increased susceptibility to RNIs in broth cultures (Guthlein et al. 2009; Darwin and Nathan 2005).

The characterization of human granuloma is an active area of research (Tsai et al. 2006). Human granulomas are generally comprised of a central core with $CD68^+$ epithelioid cells, surrounded by macrophages, T lymphocytes (predominantly $CD4^+$), and multi-nucleate giant Langerhans cells. Another characteristic feature of human granulomas, which is different from murine granulomas, is the presence of central acellular eosinophilic region formed due to necrosis and apoptosis of cells. Because this central region does not possess any vascularization, the environment surrounding these cells becomes hypoxic (Aly et al. 2006). Proteome profiling, microarray analysis, and molecular genetic studies have shown that *M. tuberculosis* survives within granuloma by varying the expression levels of DNA repair genes, the expression of some of these genes were either upregulated or downregulated significantly under hypoxic conditions (Gorna et al. 2010; Kim et al. 2008; Saunders and Britton 2007; Bacon et al. 2004; Voskuil et al. 2004; Muttucumaru et al. 2004; Smeulders et al. 1999). In vitro studies show that *M. smegmatis* strains deleted for *ung* and *uvrB* genes display increased sensitivity

to hypoxia (Kurthkoti et al. 2008; Kurthkoti and Varshney 2012). Studies with artificial granulomas of mice based on hollow-fibre model revealed that *uvrA* and *recF* were upregulated during dormancy stages (Karakousis et al. 2004). Mice exposed to aerosolized virulent strain of *M. tuberculosis* develop persistent infection and become chronic after 45–60 days post-infection. Transcriptional profiling during chronic and reactivation phases of murine tuberculosis using in vivo microarray analysis (IVMA) shows that even after 28 days of aerosol infection, CFU counts were not changed, although the bacilli were metabolically active with a 50% active transcriptome. A total of 137 genes were significantly regulated in mid-chronic tuberculosis (45 and 60 days) compared to an early stage (14 days) where the expression of genes involved in lipid and carbohydrate metabolism was significantly enriched. Additional genes, including the virulence regulator *virS* and the DNA repair protein *ung*, were upregulated during the reactivation stage, and *hupB* and *recO* were downregulated, indicating their possible roles in mycobacterial resurgence (Talaat et al. 2007). Altogether, these findings along with genome-wide expression analyses from human lung samples indicate that, while residing in granulomatous tissue, *M. tuberculosis* undergoes several changes in the expression of DNA repair genes.

The reactivation of infection begins with the disintegration of granulomas because of the failure of macrophages or T cells and with the decline in the host's immunity. Although little is known about reactivation of infection, various studies based on the effect of gene and/or protein expression levels or their activity on survival implicate a role for mycobacterial DNA repair genes. In vivo studies with immunocompromised BALB/C mice suggested upregulation in the expression levels of *M. tuberculosis* genes *ogt*, *dinG*, *ung*, and *recA* during reactivation. Of note, even though the gene expression profiles of all isolates were found to be similar, the profiles of upregulated DNA repair genes were different in granuloma, pericavity, and distant lung tissue (Talaat et al. 2007). The observed differences could be due to dissimilar physiology of cells; thus additional studies are needed to understand the complexity and reactivation of infection mounted by this respiratory pathogen.

16.7 *Mycobacterium tuberculosis* and Chemotherapy

The continuous exposure to the antibiotics leads to the emergence of drug resistance strains in *M. tuberculosis* (Karunakaran and Davies 2000; Martinez and Baquero 2000). Drug resistance strains can be divided into two subgroups: MDR (multidrug-resistant) and extensively drug-resistant (XDR) strains. These notations are based on the fact that MDR strains are resistant to two front-line drugs, isoniazid and rifampicin, whereas XDR strains are resistant to isoniazid and rifampicin including fluoroquinolones and second-line drugs (capreomycin, kanamycin, or amikacin). In general, antibiotic resistance is thought to arise from horizontal gene transfer carried by plasmids or transposons and random mutations in the chromosomal genes (Davies 1997). The genome-wide studies have indicated that the occurrence of

drug-resistant strains is due to the generation of spontaneous mutations or gene inactivation (Cubillos-Ruiz et al. 2008; Filliol et al. 2006; Tang et al. 2005; Arnold et al. 2005). For instance, resistance towards anti-tubercular agents has been linked to SNPs in drug target genes, including *rpoB*, *katG*, and *uvrB* (Eldholm et al. 2014; Ramaswamy et al. 2003; Zaczek et al. 2009). Like all other organisms, DNA repair genes directly influence the mutation frequency in mycobacteria. For example, deletion of *ung*, *uvrB*, *uvrD*, *fpg*, and *udgB* in *M. smegmatis* and *ada/alkA* together with *ogt* in *M. tuberculosis* leads to increase in mutation rates (Dos Vultos et al. 2009). In view of these results, it was speculated that the use of antibiotics in combination with new drugs targeting the DNA repair genes might increase the effectiveness of chemotherapy. For example, it was found that *recA*-deficient mutant of BCG had increased susceptibility to metronidazole in vitro and an *ogt* mutant of BCG had increased susceptibility to isoniazid (Wiid et al. 2002; Sander et al. 2001). However, with the use of each drug, it would be important to ascertain that targeting DNA repair proteins do not result in an increased mutation rate that could lead to the emergence of drug-resistant strains (Gorna et al. 2010).

16.8 Why Study NER in *Mycobacterium tuberculosis*?

Although much is known about NER in *E. coli*, gaps remain in our understanding of this pathway in other bacteria. Several studies have provided experimental evidences that the damage search/recognition complex is comprised of two each of UvrA and UvrB subunits, but exactly how these subunits distinguish damaged from undamaged DNA remains unknown. Among many unanswered questions, the oligomeric status of UvrB and the nature of the preincision complex are unclear. Do two UvrB subunits bind at the site of a lesion? If UvrB exists as a dimer in solution, then what is the mechanism underlying the formation of $UvrA_2B_2$ complex as seen in *Geobacillus stearothermophilus* crystal structure? In this structure, the two UvrB subunits are located ~145 Å apart from each other and positioned ~80 Å away from the lesion (Pakotiprapha et al. 2012). This raises another question: which UvrB subunit will remain bound to the lesion during the successive steps of NER? Further, how UvrA dimer specifically transfers damaged strand to UvrB? How damage is detected among structurally diverse lesions and substrates by $UvrA_2B_2$? How UvrA is dislodged from the repair complex and how does UvrB recruit UvrC? What is the stoichiometry of UvrB and UvrC in the complex? What is the functional relevance of UvrC homodimers and homotetramers? There exist significant differences in the NER pathway between *E. coli* and other bacteria, but very less is known about NER, especially in bacterial pathogens such as *M. tuberculosis*. Furthermore, many species like *M. smegmatis*, *Streptomyces* species, *D. radiodurans*, and *Pseudomonas putida* possess a second copy of UvrA, but its actual role in vivo is unclear (Timmins et al. 2009; Tark et al. 2008).

The deletion of *Yersinia pseudotuberculosis* and *Borrelia burgdorferi uvrA* genes or disabling UvrA activity leads to their decreased virulence (Darwin and Nathan 2005; Garbom et al. 2004; Graham and Clark-Curtiss 1999). Additionally, the *uvrA*

gene transcript levels are upregulated in *M. tuberculosis* grown in human macrophages (Graham and Clark-Curtiss 1999). Trans-complementation of *M. tuberculosis uvrB* demonstrated a crucial role in mounting resistance against nitrosative, oxidative, and UV exposure-induced DNA damage (Darwin and Nathan 2005; Graham and Clark-Curtiss 1999). *M. tuberculosis uvrB* has been shown to be important for attenuation of virulence in iNOS wild-type and iNOS knockout mice (Darwin and Nathan 2005). Notably, clinical isolates of XDR strains of *M. tuberculosis* harbour a unique $uvrB^{A582V}$ mutation, an important (catalytic) active site residue (Eldholm et al. 2014). An inhibitor, [2-(5-amino-1,3,4-thiadiazol-2-ylbenzo[f]chromen-3-one) (ATBC)], against UvrABC complex works at a micromolar concentration, but the molecular basis of its activity and the specific target within the complex has not been deciphered (Mazloum et al. 2011). Thus, efforts are underway to identify *M. tuberculosis* key determinants of infection and vulnerable targets from the NER system whose structures and biochemical functions could be exploited for the development of new antitubercular agents (Ferraris et al. 2018).

Genetic analysis revealed that *M. tuberculosis* UvrA is important for survival against DNA-damage, including nitrosative and oxidative DNA damage, implicating this gene in the persistence of the bacillus in the host. Comparative analysis of the crystal structures of *M. tuberculosis* UvrA in its ligand free form with *B. stearothermophilus* UvrA•UvrB complex, *B. stearothermophilus* UvrA•ADP, and *Thermotoga maritima* UvrA•DNA showed the conformational plasticity displayed by UvrB binding domain of UvrA (Rossi et al. 2011). Altogether, combined genetic, biochemical, bioinformatics, and structural investigations of *M. tuberculosis* UvrA have provided significant insights into the mechanistic details of UvrA from *M. tuberculosis.* In-depth biochemical characterization revealed that the *M. tuberculosis* UvrB subunit behaves as a DNA-stimulated ATPase also possessing an ATP-dependent DNA helicase activity (Fig. 16.5) (Thakur et al. 2016). Recent structural and biochemical studies have demonstrated direct interaction between *M. tuberculosis* UvrA and UvrB proteins in the absence of any ligands and further suggested that this protein–protein interaction may be exploited as a target for blocking NER in *M. tuberculosis* (Ferraris et al. 2018; Lahiri et al. 2018; Singh 2017). Although biochemical and structural studies are limited in the case of *M. tuberculosis* UvrC, it is identified as an essential gene for survival in mouse model of infection in transposon mutagenesis screen, and mutations in this gene result in increased sensitivity against UV and hydrogen peroxide (Sassetti et al. 2003; Prammananan et al. 2012).

M. tuberculosis possesses two UvrD homologues: UvrD1 and UvrD2 (Cole 1998). *M. tuberculosis* UvrD1 is a DNA helicase with 3′–5′ polarity and unwinds nicked DNA substrates resembling NER intermediates but displays maximum DNA unwinding activity on replication forks (Curti et al. 2007; Singh et al. 2010). The strains in which *uvrD1* was inactivated either alone or in conjunction with *uvrA* showed increased susceptibility to UV irradiation, mitomycin C, RNI, and ROI. Moreover, *uvrD1* and *uvrA uvrD1* mutants showed attenuation following infection of mice either by aerosol or intravenous route (Houghton et al. 2012). *M. tuberculosis* UvrD1 and UvrA also suppress DNA strand exchange catalysed

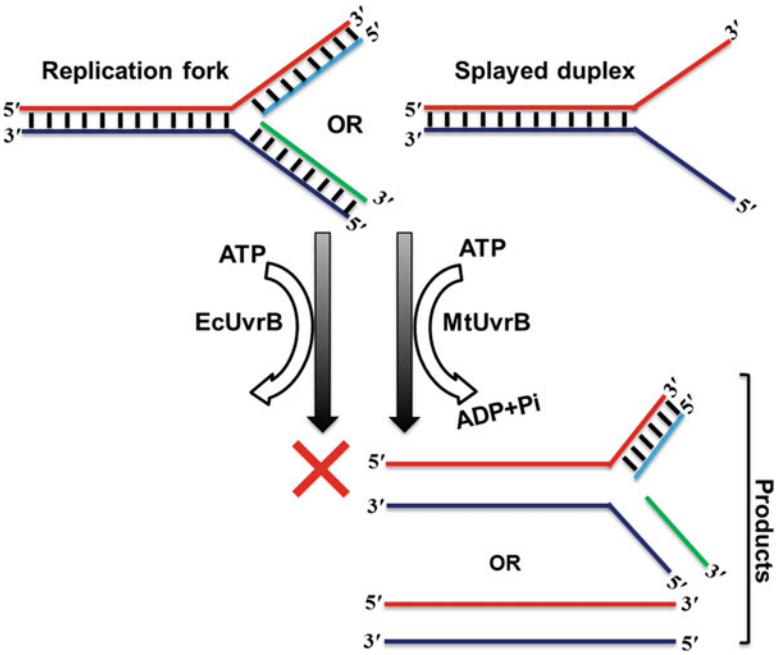

Fig. 16.5 Model depicting differences in the biochemical properties of MtUvrB and EcUvrB subunit. Each line (red and blue colour) represents a DNA strand with polarity as indicated. Experimental evidences support the notion that MtUvrB can unwind DNA replication intermediates like splayed duplex or replication fork DNA structures in an ATP-dependent manner. In contrast, EcUvrB neither hydrolyses ATP nor unwinds these structures of its own. EcUvrB needs EcUvrA to activate its ATPase as well as helicase activity. (Figure adapted with permission from Thakur et al. 2016) [Copyright 2016 American Chemical Society]

by *M. tuberculosis*, *M. smegmatis*, and *E. coli* RecA suggesting molecular cross talks with homologous pathway involved in chromosome stability (Singh et al. 2010). Inactivation of *uvrB* and *uvrD1* genes increased marker integration frequencies, further suggesting a role for these proteins in other DNA transaction processes beside NER (Guthlein et al. 2009). Additionally, mycobacterial Ku interacts with UvrD1 in a genome-wide yeast two-hybrid screen and stimulates UvrD1 to catalyse its ATP-dependent unwinding of 3′-tailed DNA. This interaction was further supported by the formation of a stable ternary complex of UvrD1, Ku, and DNA in the absence of ATP (Sinha et al. 2007). Altogether, these findings revealed structural and functional cross talk between NHEJ and NER. UvrD2 is the product of an essential gene which is comprised of N-terminal ATPase domain (Pfam00580) typical of superfamily I helicases and C-terminal HRDC domain (Pfam00570) typical of superfamily II helicases. These two domains are connected by a CxxC-(14)-CxxC tetracysteine module, which is present in helicases from *Actinomycetales* (Sinha et al. 2007). The C-terminal HRDC domain is not required for in vitro DNA helicase and ATPase activity, whereas tetracysteine module (but not the

tetracysteines) is required for DNA unwinding. Surprisingly, the helicase activity of a truncated UvrD2 lacking the tetracysteine and HRDC domain can be restored by the addition of Ku protein (Sinha et al. 2007). These findings suggest that the Ku-dependent unwinding activity together with unusual domain structure of UvrD2 may have a biological function, distinct from that of *M. smegmatis* UvrD1. In *M. tuberculosis*, complementation by N-terminal domain of UvrD2 was able to compensate for the loss of *uvrD2* deletion suggesting that the C-terminal domains are not essential. Moreover, an ATPase dead form of UvrD2 protein was unable to translocate along DNA and cannot compensate for lack of the wild-type protein. These studies indicate that the role of UvrD2 is not to unwind DNA, but rather in DNA translocation and protein displacement (Williams et al. 2011). Since mycobacterial genome is rich in GC content, it is predicted that many genes involved in persistence and pathogenesis have G-quadruplex forming motifs in their promoter region, which might regulate their expression (Perrone et al. 2017). More recently, MtUvrD1 and MtUvrD2 were subjected to extensive in vitro biochemical studies, which revealed that these proteins have the potential to resolve G-quadruplex structures, implicating their important roles in maintenance of genome integrity and gene expression via G-quadruplex DNA resolution (Saha et al. 2019). The *polA* mutants of *M. smegmatis* displayed hypersensitivity to DNA damage induced by UV irradiation and hydrogen peroxide. Using transposon site hybridization (TraSH), it was found that *polA* gene is required for the optimal growth of *M. tuberculosis* (Gordhan et al. 1996; Sassetti et al. 2003). Mycobacteria possess *ligA* (encodes a NAD(+)-dependent enzyme), which is essential for its viability (Korycka-Machala et al. 2007; Srivastava et al. 2005). In addition, *M. tuberculosis* contains three additional ATP-dependent ligases, LigB, LigC, and LigD which are shown to be nonessential for cell growth under in vitro conditions (Gong et al. 2004). Interestingly LigD interacts with Prim-PolC in the presence of single nucleotide gap containing dsDNA substrate. Deletion of *ligD* gene was found to be sensitive to oxidative damage, suggesting the role of LigD in the excision repair other than NHEJ.

16.9 Concluding Remarks

M. tuberculosis, an exquisitely adapted human pathogen, encounters hostile environments during transmission, infection, and clinical latency. *M. tuberculosis* encodes a complex array of enzymes of several lipid pathways, DNA metabolic pathways, cell surface proteins, and regulators, which are essential for genome stability and cell survival in infected host cells. The attenuation of virulence of *M. tuberculosis uvrB , uvrA*, and *uvrD1* mutant strains in mice suggests a possible role for NER in pathogenesis. Although the genes encoding proteins involved in the NER pathway are conserved between E. coli and M. tuberculosis, the observation that NER components in tubercle bacillus exhibit significantly different mechanistic properties from the E. coli UvrABC proteins is worthy of further investigation. Additionally, it would be interesting to investigate the role of NER in the generation

of MDR and XDR strains of *M. tuberculosis* and phenotypic heterogeneity among these strains. These studies might help to guide the development of more efficacious treatments targeting the NER components of *M. tuberculosis.* In conclusion, we envision that further research on the role(s) of NER components in tubercle bacillus may provide insights into the processes critical for genome maintenance and new opportunities for therapeutic intervention.

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