



Exploiting Bacterial DNA Repair Systems as Drug Targets: A Review of the Current Scenario with Focus on Mycobacteria

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Abstract | Bacterial DNA repair systems as a drug target have been drawing increasing attention. Recent research has highlighted important differences between bacteria and humans in these systems. Additionally, most existing drugs are not known to act through inhibition of the components of these systems, especially involving the principal replicative ligase, and hence afford the possibility to develop new therapeutics that can overcome present problems of drug resistance. Recent work has highlighted significant differences in DNA repair systems between mycobacteria and others like *E. coli*, leading to the possibility of developing inhibitors that can distinguish even between these bacteria. In this review, present information on these systems from the point of view of new antibacterial development in general is collated. The subsequent focus is on mycobacterial DNA repair systems, particularly those involving DNA ligases. Presently available inhibitor scaffolds and new approaches for the development of potent inhibitors are also discussed.

Keywords: *MtbLigA*; *NAD*⁺-dependent DNA ligase from *M. tuberculosis*; *BER*; Base-excision repair pathway; *NER*; Nucleotide excision repair pathway; *NHEJ*; Non-homologous end-joining pathway; *HR*; Homologous recombination.

1 Introduction

The historic milestone in the fight against pathogenic bacteria is arguably the breakthrough discovery of penicillin in 1928. This was followed by numerous discoveries of additional classes of antibiotics that form the current arsenal of antimicrobials. Excessive use/misuse of antibiotics during the past years has unfortunately led to many pathogenic bacteria mutating into drug resistant strains. Bacteria exhibit diverse mechanisms of drug resistance against formerly successful drugs, a phenomenon called ‘multiple drug resistance’ (MDR), which is extremely difficult to treat.^{1,2} There are several strains that have gained resistance against all available agents, eg. MRSA (Methicillin-Resistant *Staphylococcus aureus*), a gram-positive bacterium, which is not only resistant to methicillin but also aminoglycosides, macrolides, tetracycline, chloramphenicol, and lincosamides.³ A big threat also comes from gram-negative bacteria like

Acinetobacter and *Pseudomonas* spp which have acquired pan-drug resistance.^{4,5} Alarmingly, the emergence of resistant pathogens that is leading to increasing fatalities is accompanied by relative stagnation in the development of new antibiotics, especially those that have new modes of action. It is clear that in spite of the substantial progress in the development of modern drug discovery tools that utilize biological, chemical, informational, and technological interfaces, there have been disproportionate difficulties in the identification of new lead therapeutic compounds.^{6,7} The emergence of clinically significant resistant strains of microbial pathogens has led to an increase in the discovery/production of antibacterials, but most of them are derivatives of old traditional classes of antibiotics. Compared to only two systemic antibacterial agents approved for use in humans by the U.S. FDA from 2008 to 2013, sixteen were approved from 1983–1987. In particular, there has been no new

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class of antibiotics to treat Gram-negative bacilli (GNB) for more than 40 years. Amazingly, the fluoroquinolones were the last new class of antibiotics to treat GNB. Resistant bacteria employ varied approaches to escape antibiotic pressure, due to which there is an acute need for the development of new antibiotics with the rise of novel modes of action.

More recently, there has been an explosion of data that significantly augment our knowledge of bacterial physiology.⁸⁻¹³ Informational and technological developments have given rise to large amounts of data, especially the complete genome sequence of thousands of bacterial genomes.¹⁴ Systematic high throughput efforts in generating gene-knockouts of various bacterial species have shed light on the role and essentiality of numerous genes.¹¹ Structural genomics initiatives have led to the determination of three dimensional structures of various crucial and essential proteins that could be exploited as promising drug targets. These are expected to be helpful in structure-based new inhibitor discovery approaches.¹² Entire protein-protein network analyses have been carried out in model organisms like *Escherichia coli*.¹⁵ Studies like these lead to better understanding of the individual gene components against a systems biology background. Recent advances in technology has assisted the *in vivo* studies of proteins inside the live bacterial organism to support the decades of *in vitro* studies.¹⁶ The plethora of high-throughput information available from model organisms can now be extrapolated to human pathogens. It is small wonder that this vast knowledge and information can pave the way towards the expected development of new classes of antibiotics that utilise new modes of action and new drug targets.

Antibiotics in current use mostly target a diminutive number of bacterial targets, most of which affect peptidoglycan, biosynthesis or gene expression/translation.¹⁷ These targets are either essential for growth and propagation of pathogens, or are absent from their human host and are conserved across wide range of bacterial pathogens.¹⁸ It is evident from enormous high throughput knock-out and sequencing data that there remain many additional bacterial cellular targets essential for pathogen survival, and which are not the targets of any of the presently available armour of antibiotics.

2 Bacterial DNA Metabolism

DNA metabolism is a fundamental process carried out by bacteria to maintain its survival. DNA metabolism involves many vital life-sustaining processes like DNA replication, DNA

recombination, DNA repair, transcription etc. These processes require a large set of proteins and protein complexes to work in a highly coordinated organized manner. The three indispensable mechanisms replication, repair and transcription share many of their functional workers following 'common enzyme-diverse pathways approach'. This strategy may be fascinating for the development of antimicrobials, as therapeutic intervention with any one critical functional component can influence more than one vital life processes in the bug, making it difficult to survive. Moreover, such inhibitors should also have broad-range activity as these modules are highly conserved. There are several reasons that lead to DNA damage in bacteria; but an important reason especially during bacterial infection involves the release of chemicals by host cells that leads to bacterial DNA damage. Such damage is highly mutagenic and cytotoxic, and if left untreated leads to bacterial cell death. At the same time if genetic material is perpetuated with perfect fidelity, the genetic variation needed to drive evolution would be lacking and new strains such as resistant forms of the pathogens would not arise. So the success story of pathogenic life depends on a happy and delicate balance between mutation and repair. The nature of mutation decides how the repair action should take place, and reverse the conceivable change in the DNA sequence. Some of the repair strategies employed by bacteria are outlined below.

2.1 Mismatch repair

The replication machinery has high degree of accuracy due to the proof reading component of the *replisome**, the 3'-5' exonuclease which removes wrongly incorporated nucleotides.

However, this proof reading is not foolproof and sometimes incorrectly added nucleotides escape detection and become a mismatch between the newly synthesized strand and template strand. Such mismatches if not detected and replaced would get lodged in the DNA permanently and lead to deleterious effect on the growth of the pathogen. A specialized mechanism of mismatch repair is reserved to deal with such genomic sequence alteration. The scanning action for detection of mismatch is performed by MutS, a protein that recognises DNA distortion. It further recruits MutL, a second component of the repair system. MutL, in turn, activates MutH, which causes an incision near the site of mismatch, followed by the unwinding of the DNA strand from the incised point by UvrD to the mismatched site. Exonuclease progressively digests the displaced strand creating a single stranded gap that is then filled by DNA

polymerase III. The nick is subsequently sealed by the action of DNA ligase. Mismatch repair systems are highly conserved in bacterial populations and have evolved from common ancestors, *i.e.* the Hex system in Gram-positive *Streptococcus pneumoniae* and the Mut system in Gram-negative *E. coli*, *Salmonella enteric*, *Serovar typhimurium*^{19,20} and *Saccharomyces cerevisiae*.²¹ But at times, the constant stress conditions that prevail in the human host, cause the repair machinery itself to get affected. Any alterations in genomic DNA that leads to mutations or loss of mismatch repair system may result in greater chances of mutations in the pathogens, which can give rise to selected resistant strains. The resistant mutants may have altered targets of a drug, reduced permeability to the antibiotic, increased efflux of the antibiotic or upregulated antibiotic-inactivating enzymes. Hypermutability arising due to loss of DNA mismatch repair systems, can be co-selected with antibiotic resistance. This means that mutants with resistance to one agent are more likely to develop subsequent resistance to unrelated agents.²² Siegel & Bryson (1967) discovered the MutS gene in an azaserine-resistant derivative of *E. coli* that had a mutator phenotype and carried a deletion in the MutS gene. The mutator phenotype has been linked in several bacterial genera to a defect in the methyl-mismatch repair system, in which the chief modules are MutS and MutL and UvrD. This system is involved both in mismatch repair and in prevention of recombination between homologous fragments in *Escherichia coli*. This has been shown to play an important role in the adaptation of bacterial populations to changing and stressful environments including the development of antibiotic resistance based on the reasons delineated here.^{23,24}

2.2 Base Excision Repair (BER)

Mutations do not arise only from errors in replication but also as a result of environmental mutagens that may be produced inside the pathogenic host as a defence immune response or outside. Most frequent mutations arise due to alkylation and oxidation of nitrogenous bases that lead to the generation of altered bases which are highly mutagenic and cytotoxic. The other harmful modifications arise from ionising radiations which are clastogenic in nature and lead to double and single-stranded DNA breaks. There are specialized systems of DNA repair present in bacteria to deal with the altered bases, namely Base excision repair (BER) and nucleotide excision repair (NER) respectively. BER involves an enzyme, glycosylase, which is a lesion specific enzyme. Cells

have multiple DNA glycosylases with different specificities for lesions like uracil generated by the deamination of cytosine or oxoG generated by oxidation of guanine like Ung, UdgB, MutM, MutY.^{25–31} DNA glycosylases diffuse laterally along the minor groove until a specific lesion is detected. They recognise the damaged base which is flipped out of the DNA double helix. The base is then removed by hydrolysing the N-glycosidic bond, resulting in the production of abasic sites. These abasic sites are highly mutagenic in nature. The abasic sites are further processed by the action of AP endonucleases that incise the DNA phosphate backbone and produce a nick. The nick is then sealed by downstream processing enzymes like DNA polymerase and DNA ligase, thereby restoring the intact strand using the undamaged strand as the template.^{32–38}

2.3 Nucleotide Excision Repair (NER)

Unlike base excision repair, the nucleotide excision repair enzymes do not recognise any particular lesion. Rather the NER system works by recognising the distortion in the shape of the double helix. The task is largely accomplished by four major proteins, *viz.* UvrA, UvrB, UvrC and UvrD. A complex of UvrA-UvrB scans along the length of DNA in an ATP-dependent manner. UvrA detects the distortion and exits leaving behind UvrB to melt the DNA creating a single stranded bubble around the lesion. This step is followed by the recruitment of UvrC that incises both sides of the lesion. This cleavage creates 12–13 residue long single-stranded DNA segments made accessible by DNA helicase, UvrD. Finally DNA polymerase I and DNA ligase seal the gap.^{39–42}

2.4 Recombination repair of DNA

Owing to constant assault by the host defence system, the maintenance of genomic integrity is a big task for a pathogen. This might give rise to a situation whereby the replication fork halts due to the presence of an unattended DNA lesion, and leaving the lesion in a single-strand gap at the stalled fork. Alternately, if the replication fork encounters a DNA strand break, a double-strand break separates one branch of the fork. Such stalled replication forks can be processed by diverse means to give rise to a DNA end with double stranded breaks. These broken DNA ends then commence recombination with homologous DNA molecule which will in turn heal the break. The cellular recombinational DNA repair system consists of flexible set of enzymes that can process whatever DNA structures might exist at a collapsed replication fork. The first set of proteins to come into

Table 1: Structural data available in the Protein Data Bank (<http://www.rcsb.org/>) for proteins in the different DNA repair pathways discussed.

Proteins	Enzymatic activities	Gene annotation in Mycobacterium Tuberculosis	Pdb ids of structures from Mycobacterial*	Pdb ids of structures from other relevant bacterial sources (organism)*.*
Mismatch Repair Pathway				
MutS	Methyl-directed mismatch repair protein/ATPase	ABSENT	-	2 WTU(Ec) 3ZLJ(Ec) 1OH5(Ec) 1EWR(Ta) 1NNE(Ta)
MutL	Methyl-directed mismatch repair protein/ DNA-stimulated ATPase	ABSENT	-	1BKN(Ec) 3GAB(Bs)
MutH	Methyl-directed mismatch repair endonuclease	ABSENT	-	1AZO(Ec) 2AZO(Ec)
UvrD	ATP-dependent 3'-5' DNA helicase II	ABSENT	-	3LFU(Ec) 2IS2(Ec)
RecJ	ssDNA 5'-3' exonuclease	ABSENT	-	2ZXO(Tt)
BER/NER Pathway				
Ung	Class 1 Uracil DNA glycosylase.	Rv2976c	3A7N	3UF7(Ec) 1LQJ(Ec) 2HJQ(Bs)
Udg	Class 5 Uracil DNA glycosylase	Rv1259	-	
Fpg	Formamidopyrimidine DNA glycosylases	Rv2924	-	1K82(Ec) 3TWL(At)
Fpg2	Formamidopyrimidine DNA glycosylases	Rv0944	-	
Nei1	Endonuclease VIII	Rv2464c	-	2EAO(Ec) 2OPF(Ec) 2OQ4(Ec)
Nei2	Endonuclease VIII	Rv3297	-	
Nth	Endonuclease III	Rv3674	-	2ABK(Ec) 1P59(Gs)
MutY	Adenine DNA glycosylase	Rv3589	-	1WEF(Ec) 1KG2(Ec)
TagA	Glycosylase	Rv1210	-	
AlkA	Methylphosphotriester-DNA-protein-cysteine S-methyltransferase	Rv1317	-	1DIZ(Ec) 3OH6(Ec)
Mpg	Glycosylase	Rv1688	-	2ZU7(Ph)
Nfo	Endonuclease IV	Rv0670	-	4K1G(Ec) 2NQ9(Ec) 1XP3(Ba)
Xth	Exonuclease III	Rv0427	-	1AKO(Ec) 2JC4(Nm) 2VOA(Af)
Dut	dUTPase and dCTPase activities	Rv2697c	1SM8 1SMC 2PY4 4GCV ISLH 3LOJ 1SIX	1SEH(Ec) 1EU5(Ec) 1EUW(Ec)
MutT1	8-oxo(dGTP)/8-oxo(GTP) Hydrolase, dGTP, dTTP hydrolase	Rv2985	-	3A6S(Ec) 2PQV(Sp) 3HHJ(Bh) 3DUP(Rr)

(Continued)

Table 1: (Continued).

Proteins	Enzymatic activities	Gene annotation in Mycobacterium Tuberculosis	Pdb ids of structures from Mycobacterial*	Pdb ids of structures from other relevant bacterial sources (organism)#,*
MutT2	8-oxo-dGTPase activity	Rv1160	-	
MutT3	dNTPases/NTPases	Rv0413	-	
MutT4	dNTPases/NTPases	Rv3908	-	
RdgB	NTPase for removal of hypoxanthine/xanthenes triphosphates	Rv1341	-	1K7K(Ec) 2PYU(Ec) 2Q16(Ec)
MazG	nucleoside triphosphate pyrophosphohydrolases	Rv1021	-	3CRA(Ec) 3CRC(Ec) 2YF9(Dr)
UvrA	ATPase/excinuclease	Rv1638	3ZQJ	
UvrB	Excinuclease/Stimulates ATPase activity of UvrA	Rv1633	-	1QOJ(Ec) 1T5L(Bc) 2D7D(Bs) 2NMV(Bs) 3V4R(Bs)
UvrC	Excinuclease/Attaches to UvrA-B and incises the damaged DNA on both sides of the damaged site	Rv1420	-	1KFT(Ec) 3C65(Bst)
Mfd	Involved in transcription coupled repair	Rv1020	-	2EYQ(Ec) 2Q5R(Sp), 3HJH(Ec) 2B2N(Ec)
UvrD1	3'-5' DNA helicase	Rv0949	2LQQ	3LFU(Ec) 2IS1(Ec)
UvrD2	3'-5' DNA helicase	Rv3198	-	
XPB	3'-5' DNA helicase	Rv0861	-	2FWR(Af) 2FZL(Af) 2FZ4(Af)
PolA	DNA polymerase	Rv1629	-	3HQA(Ec) 1DPI(Ec) 3EYZ(Gb) 4ELT(Ta)
DinP	Y-family DNA polymerases V	Rv3056	-	4K74(Ec)
DinX	Y-family DNA polymerases IV	Rv1537	-	
DnaE2	error prone DNA polymerase	Rv3370	-	1ZDE(Ss)
LigA	NAD ⁺ dependent DNA Ligase	Rv3014c	1ZAU(adenylation domain) 3SGI(BRCT deleted LigA)	2OWO(Ec) 3JSN(Sa) 3BAC(Hi) 1DGS(Tf) 1V9P(Tf)
ligB	ATP dependent DNA Ligase	Rv3062	-	3GDE(Af)
LigC	ATP dependent DNA Ligase	Rv3731	-	
LigD	ATP dependent DNA Ligase. Involved in NHEJ	Rv0938	1VSO(ligase domain) 2IRU(polymerase domain) 2R9L(polymerase domain)	2FAR(Pa) 3N9B(Pa) 2FAO(Pa)

(Continued)

Table 1: (Continued).

Proteins	Enzymatic activities	Gene annotation in Mycobacterium Tuberculosis	Pdb ids of structures from Mycobacterial*	Pdb ids of structures from other relevant bacterial sources (organism) ^{#,*}
Recombination Repair				
RecA	DNA dependent ATPase catalysing strand invasion and strand exchange during homologous recombination	Rv2737c	1G19 31GD	1UBC(Ms) 1NO3(Ec) 1REA(Ec) 1XMS(Ec) 2REB(Ec)
RecB	DNA helicase/nuclease	Rv0630c	-	1W36(Ec) 3K70(Ec)
RecC	DNA helicase/nuclease	Rv0631c	-	
RecD	DNA helicase/nuclease	Rv0629c	-	
RuvA	Holliday junction DNA helicase	Rv2593c	2H5X 2ZTE	1C7Y(Ec) 1D8L(Ec) 1HJP(Ec) 1CUK(Ec) 1BVS(Ml)
RuvB	Holliday junction DNA helicase	Rv2592c	-	1HQC(Tt) 1IN4(Tm) 1IN7(Tm)
RuvC	Holliday junction resolving endonuclease	Rv2594c	-	1HJR(Ec) 4EP4(Tt) 4LD0(Tt)
RecG	ATP dependent DNA helicase	Rv2973c	-	1GM5(Tm)
RecF	ssDNA and ATP binding	Rv0003	-	2O5V(Dr)
AdnA	Helicase/nuclease	Rv3202c	-	3U44(Bs) 3U4Q(Bs)
AdnB	Helicase/nuclease	Rv3201c	-	

Organism designation: Ec, *Escherichia coli* K-12; Ta, *Thermus aquaticus*; Bs, *Bacillus subtilis*; Tt, *Thermus thermophilus*; At, *Arabidopsis thaliana*; Gs, *Geobacillus stearothermophilus*; Ph, *Pyrococcus horikoshii*; Ba, *Bacillus anthracis*; Nm, *Nisseria meningitides*; Af, *Archaeoglobus fulgidus*; Sp, *Streptococcus pneumoniae*; Bh, *Bartonella henselae*; Rr, *Rhodospirillum rubrum*; Dr, *Deinococcus radiodurans*; Bc, *Bacillus caldotenax*; Bst, *Bacillus stearothermophilus*; Ta, *Thermus Aquaticus*; Gb, *Geobacillus kaustophilus*; Ss, *Synechocystis* sp; Sa, *Staphylococcus aureus*; Hi, *Haemophilus influenzae*; Tf, *Thermus filiformis*; Pa, *Pseudomonas aeruginosa*; Ms, *Mycobacterium smegmatis*; Ml, *Mycobacterium leprae*; Tt, *Thermus thermophilus*; Tm, *Thermotoga maritima*.

*** Codes shown are Protein Data Bank accession number.**

- Structure of the corresponding protein is not available.

play in recombination repair is RecBCD helicase/nuclease trimolecular complex of three subunits. It binds to the DNA molecule at double stranded breaks and unwinds the DNA strands along the length of DNA using energy from ATP hydrolysis, accompanied with or without nucleolytic degradation of one or both the DNA strands. The activities of RecBCD are controlled by specific Chi DNA sequences (GCTGGTGG). The Chi sites stimulates frequency of homologous recombination by assisting in the creation of 3'-single stranded DNA ends by altering the polarity of nuclease activity of the enzymatic complex.⁴³ The single stranded DNA tail generated by RecBCD must be coated by RecA protein for recombination to occur, which is recruited by the enzyme complex itself. RecA is a strand exchange protein that catalyses the pairing of homologous DNA molecules. The active

form of RecA is the protein-DNA filament. RecA binds readily to single-stranded DNA, nucleating and then assembling unidirectionally (5'-3') into a nucleoprotein filament. It is within the RecA filament that the search for the homologous DNA sequences is conducted and execution of DNA strands occur.^{44,45} After the strand invasion step of recombination is complete, the two recombining DNA molecules are connected by DNA branch called Holliday junction. Holiday junction specific protein RuvA recognizes and binds to the junction and recruits helicase RuvB that provides energy for the exchange of DNA pairs and move DNA branch. RuvC is the major holliday junction resolving endonuclease that further brings about cleavage and resolution of junction in complex with RuvA and RuvB by nicking two of the homologous DNA strands. The 3'-OH and

5' phosphate ends are created that are then further ligated by DNA ligase.

Recombination-based DNA repair proteins, particularly the RecBCD and AddAB helicase-nucleases, are potential targets for development of a new classes of anti-bacterial agents. These proteins are the constituents of the major pathway of DNA repair, broadly dispersed in bacteria and apparently missing from eukaryotes. They are particularly required during infection by several diverse pathogens, and are responsible for the induction of mutations that impart resistance to available antibiotics. Drugs against these enzymes should self-limit the progress of bacterial resistance. Many recent studies have resulted in promising results to exploit these yet untouched novel cellular targets. The hexapeptide WRWYCR has been recently identified as an inhibitor of bacteriophage lambda integrase (Int)-mediated site-specific recombination. The most effective peptides were found to be specific for the branched DNA structure itself, in contrast to the integrase complex. These peptides have been found to be inhibitory to several Holliday junction processing enzymes *in vitro* that includes RuvABC junction resolvase complex.^{46,47} Inhibition of recombination repair constitutes a novel target for antibiotic therapy and needs further investigation.

3 The DNA Repair Systems of Mycobacteria: A Tool for Evolution/Adaptation?

Mycobacterium tuberculosis is a major global threat to the human population. The co-infection of TB with HIV increases the risk of death twice as compared to patient with HIV infection alone⁴⁸ (WHO 2010). The integrity of the mycobacterial genome is protected by dedicated DNA repair machinery, which is of paramount importance for its survival within the host. Availability of the genome sequences from a variety of mycobacterial strains and variants has significantly advanced our knowledge of DNA repair mechanisms in this organism.⁴⁹ A growing body of evidence suggests that these pathogens often behave differently even from other bacteria like *E. coli* and *B. subtilis* especially in some of the important processes involving DNA repair.⁵⁰ The *M. tuberculosis* genome contains many of the base excision and nucleotide excision repair genes found in *E. coli*. However, it lacks homologs of the mismatch repair pathway genes.^{51,52} Interestingly, the bacterium possesses ERCC3 (XPB) and Mpg, enzymes which were until then found exclusively in mammalian cells.⁴⁹ The bacterium also encodes homologs of the non homologous end joining pathway (NHEJ),

which has been established as a major pathway for repairing double strand DNA breaks in eukaryotes. Proteins such as Ku70, Ku80, DNA ligaseD that function in this pathway have been identified in *M. tuberculosis* and other selected bacterial members.^{53,54} A similar NHEJ process is notably absent in bacteria, like *E. coli*. The capacity of *M. tuberculosis* to adapt to the ever-hostile and varying host conditions has been shown by the emergence of multidrug resistant strains (resistant to both isoniazid and rifampicin that may or may not demonstrate resistance to any other antituberculosis drugs) and, more lately, extensively drug-resistant (strains resistant to isoniazid and rifampin as well as at least two of the six primary classes of second-line drugs, one being a fluoroquinolone and the other an injectable drug.⁵⁵ Studies by Dos Vultos *et al.* demonstrated predominant high level of variation in the nucleotide sequences encoding DNA repair proteins in the W-Beijing family of resistant strains.⁵⁶ The significant role that DNA repair machinery plays in providing adaptive capability and hence the emergence of drug resistance, comes from the study of *M. tuberculosis* W-Beijing strains, a family linked to increased risk of drug resistance. This family carries an accumulation of unique mis-sense alterations in the three putative anti-mutator genes including mutT-type genes, mutT2 and mutT4.⁵⁷ The available evidence points to the importance and link between the survival/emergence of resistant mycobacterial strains and alterations in their DNA repair, recombination and replication systems. Specific mycobacterial DNA repair systems will be detailed subsequently.

3.1 Components of Mycobacterial Base Excision Repair (BER) system

The full complement of genes that encode for homologs involved in base excision repair pathway have been reported in mycobacteria.^{49,52} The proteins encoded are highly conserved compared to homologs from other bacteria. BER is initiated by DNA glycosylases, which as mentioned earlier are lesion specific enzymes that display high specificity for damaged bases. They catalyze excision by hydrolyzing the N-glycosidic bond between the base and sugar. DNA damage that arises from oxidative stress frequently involves the oxidation of guanine resulting in the generation of 7,8-dihydro-8-oxoguanine (8-oxoG) or its derivatives (Fraga *et al.*, 1990; Steenken and Jovanovic, 1997; Farr and Kogoma, 1991; David *et al.*, 2007). The presence of 8-oxoG in the template strand results in the misincorporation of A during replication resulting in C to A (or G to T) transversions.⁵⁸⁻⁶⁰

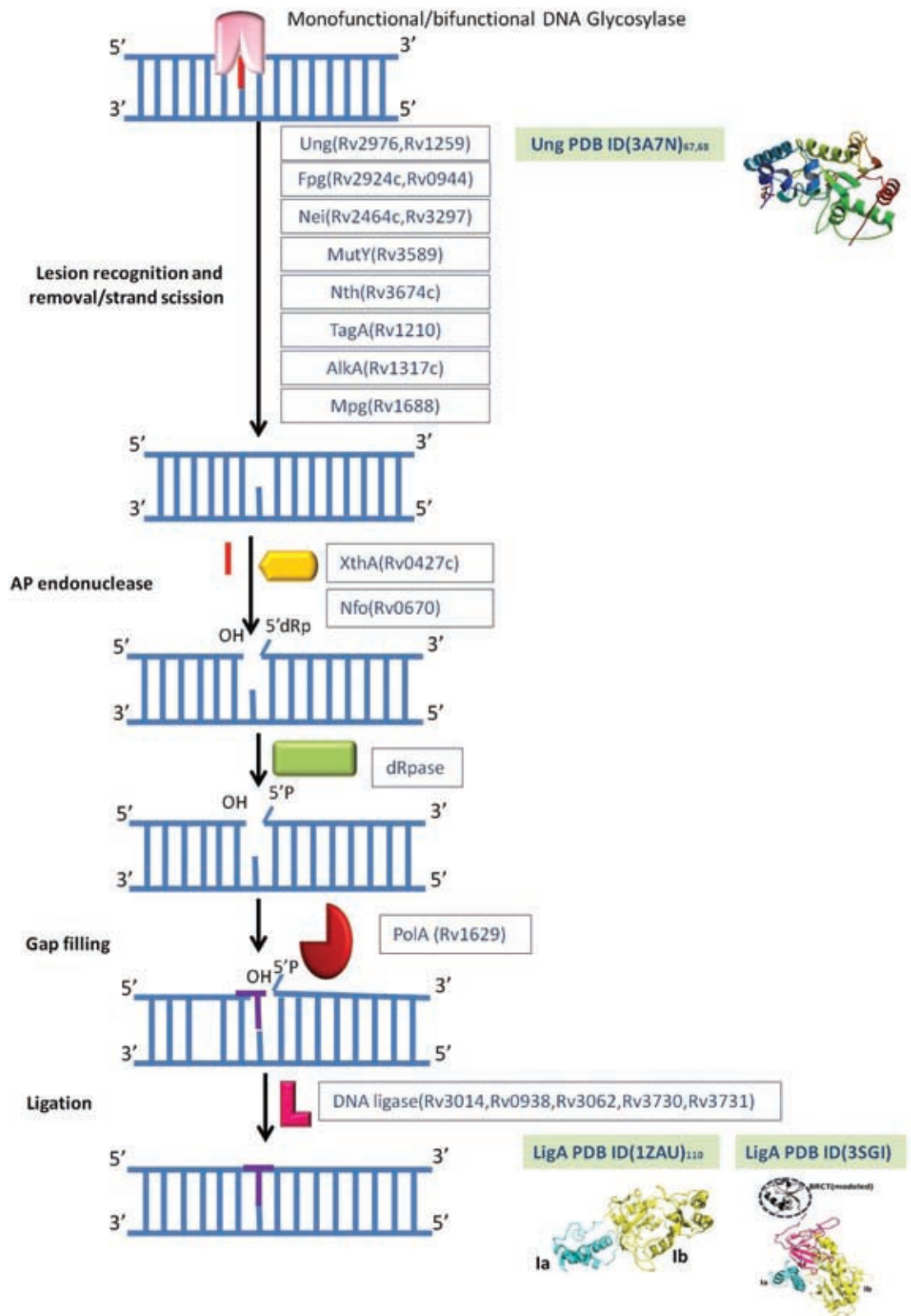


Figure 1: Schematic representation of Base Excision Repair pathway in *Mycobacterium tuberculosis*. In this and the subsequent figures, the gene annotations corresponding to the protein are shown in outlined boxes. The PDB IDs of the presently available crystal structures of the proteins involved in the pathways, with references, are in the shaded boxes.

To check such mutations, organisms have a dedicated pathway called the 'GO repair system' involving at least two enzymes to locate 8-oxoG lesions in the midst of predominantly undamaged DNA. The first one *viz.* MutM (Fpg-formamidopyrimidine DNA glycosylase), excises 8-oxoG paired against C, while the second enzyme *viz.* MutY (adenine glycosylase) is involved in removing the normal base A when paired against 8-oxoG, thus preventing the mis-incorporation of A against 8-oxoG.⁶¹⁻⁶³ The *Mpg* gene encodes for 3-methylpurine-DNA glycosylase which until then was considered to be present only in mammalian cells.⁴⁹ Mpg protein has a broad spectrum of substrates including altered bases like deaminated purines. The latter are generated as a result of the action of reactive nitrogen species produced by macrophages.⁶⁴ The lack of a mismatch repair system in *M. tuberculosis* makes it more vulnerable to cytosine deamination, although this is yet to be confirmed. The deamination of cytosine results in the incorporation of uracil in DNA which brings transversion mutations and destabilizes genomic integrity. Ung, uracil DNA glycosylase prevents such mutations by efficiently excising uracil from DNA and helps in the maintenance of the pathogen in the reactive environment of macrophages.⁶⁵ MtuUdgB excises ethenocytosine and hypoxanthine from double-stranded DNA (dsDNA) and may act as backup for Ung disabled bacteria.⁶⁶ Recent elucidation of the crystal structure of Ung from *M. tuberculosis* (PDB ID 3A7N, 2ZHX)^{67,68} demonstrated unique features of its structure and interaction. After the action of DNA glycosylases that leaves an Apurinic/Apyrimidinic (AP) site, AP endonucleases come into play to repair them. The accumulation of abasic sites are extremely mutagenic in nature as they obstruct vital life processes like replication and transcription.⁶⁹⁻⁷² Two homologs of *E. coli* AP endonucleases, *viz.* Nfo (endonuclease IV) and XthA (exonuclease III) have been reported to exist in *M. tuberculosis*. Although the protein elements of the BER pathway in mycobacteria are highly conserved they have not yet been exploited for the development of new inhibitors that target them.

3.2 Components of Mycobacterial Nucleotide Excision Repair (NER) system

The NER pathway in bacteria is an important alternative to BER, especially in the context of repair of DNA damage resulting from UV exposure such as thymine dimers, DNA cross links, strand breaks, deamination of bases, etc. generated by ROS and RNI.^{73,74} The protein components of the NER pathway in mycobacteria remains essentially

conserved in all mycobacterial strains as also in bacteria like *E. coli*. The components mainly consist of the excinuclease ABC and the DNA helicase II, *i.e.* *uvrA*, *uvrB*, *uvrC* and *uvrD* respectively. This indicates the significant importance that the NER system carries in preserving the genomic integrity. Studies have highlighted the increase in the expression of *UvrA* and *UvrB* in *M. tuberculosis* on exposure to hydrogen peroxide and amplification in the production of NER pathway gene transcripts in human macrophages.^{75,76} The *UvrB* mutant of *M. tuberculosis* was shown to be sensitive to acidified sodium nitrite. Further exploration demonstrated that the mutant exhibited deficiencies for survival within mouse.^{77,78} Very recently the crystal structure of *UvrA* from mycobacterial origin was reported, which has led to additional understanding about its reaction mechanism and mode of binding to *UvrB* binding domain.⁷⁹ The available information suggests that mycobacterial NER can prove to be a useful drug target. In fact, a recent study demonstrated therapeutic targeting of the mycobacterial NER pathway by a chemical inhibitor 2-(5-amino-1, 3, 4-thiadiazol-2-ylbenzo[f]chromen-3-one (ATBC) at micromolar concentrations.⁸⁰ However, further investigations are required to exploit this repair system as a novel target.

3.3 Components of the Mycobacterial recombination repair system

The continuous stress under which mycobacteria exists inside the host's macrophage, subjects its genomic integrity to a high degree of threat. Maintenance of genome integrity is pivotal for cell survival, yet the outcome of constant host assaults, such as double-strand breaks (DSBs) occur routinely. Such DSBs are induced by endogenous sources such as the reactive and genotoxic environment present inside the macrophage, replication across nicks etc. Double-strand breaks (DSBs) are a specific type of DNA lesions that may act as obstacles during DNA metabolism, *eg.* when replication forks encounter single-strand breaks.⁸¹ Understanding of the processes and sequence of events involved in recombination repair has largely been based on studies done in *E. coli*.⁸²⁻⁸⁴ Nature has evolved two general approaches for repairing DSBs: homologous recombination (HR) and non-homologous end joining (NHEJ) respectively. Homologous recombination is a template (homologous donor DNA molecule) dependent process which is relatively error free and occurs by either joining the two opposed ends of the broken DNA molecule or restarting the replication process from a detached DNA end. In both the cases, exchange of DNA strands occurs by a

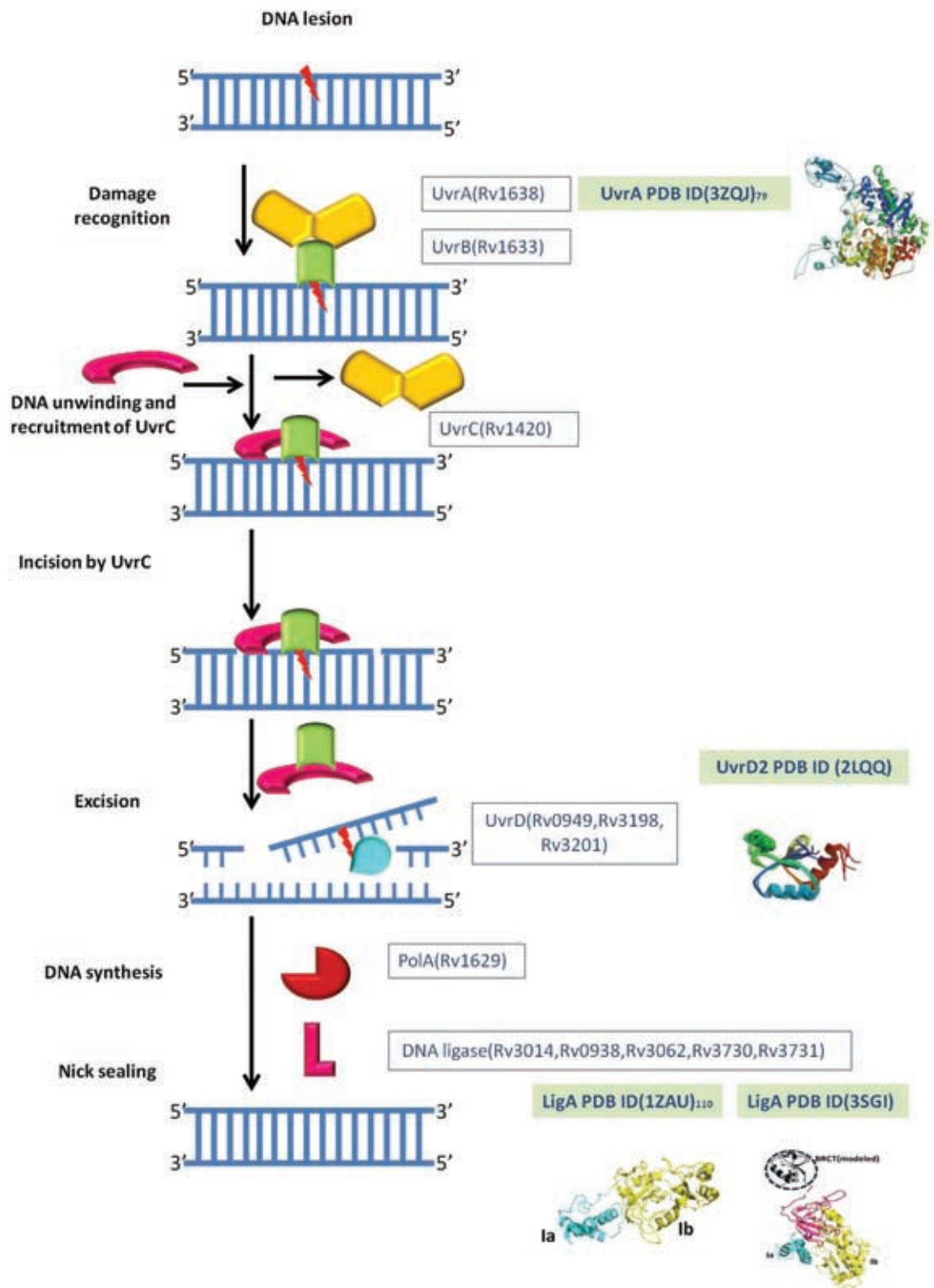


Figure 2: Schematic representation of Nucleotide Excision Repair pathway in *Mycobacterium tuberculosis*

process called synapsis ('synapsis' broadly refers to the pairing of two recombining DNA molecules) between homologous DNA molecules.^{85–87} The chief player in the recombination process is DNA-

dependent ATPase called RecA, that searches for homology and brings about strand invasion culminating in strand exchange. Consequently, DNA lesions requiring recombinational repair must first

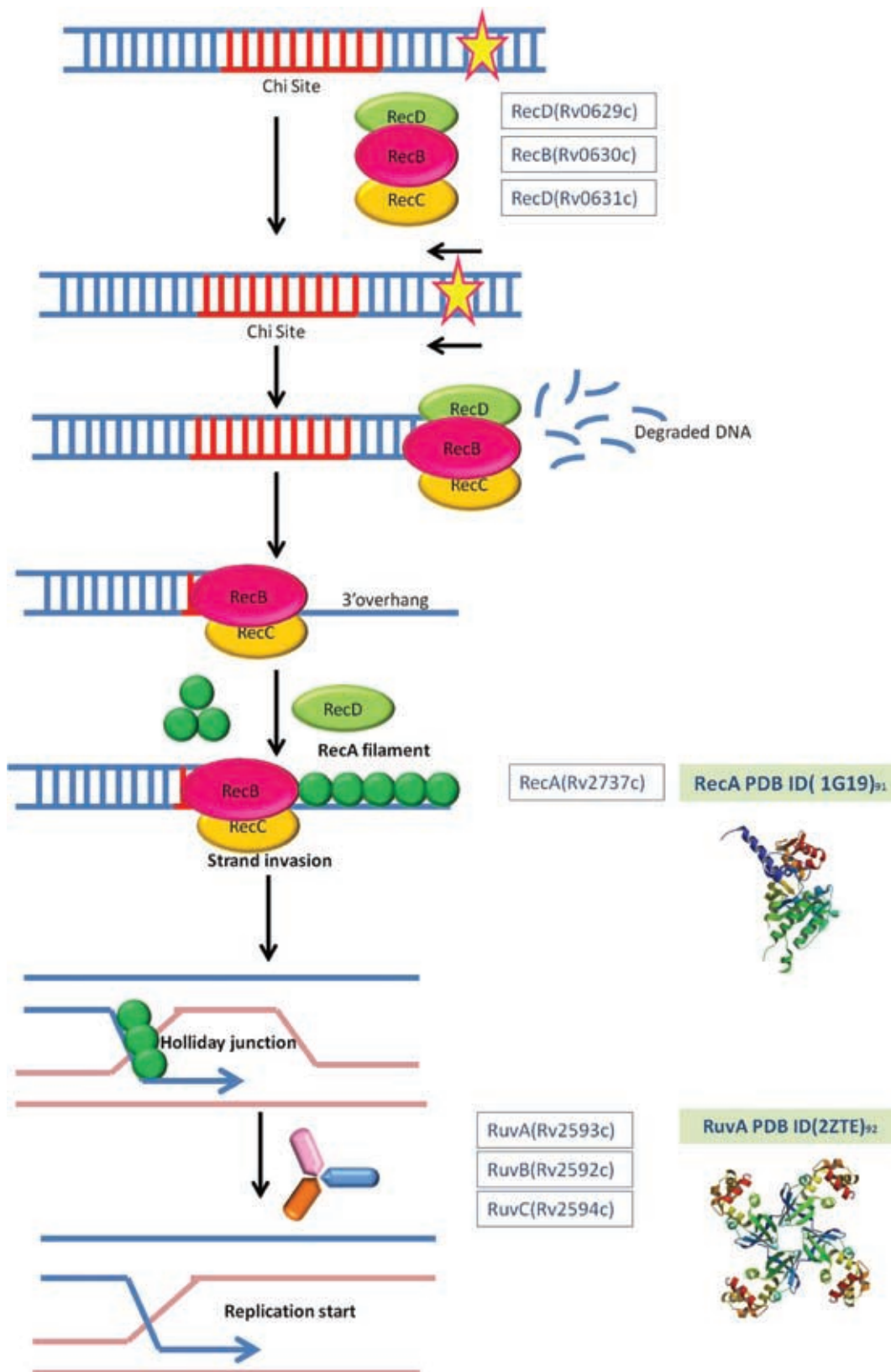


Figure 3: Schematic representation of Homologous recombination by the RecBCD pathway in *Mycobacterium tuberculosis*.

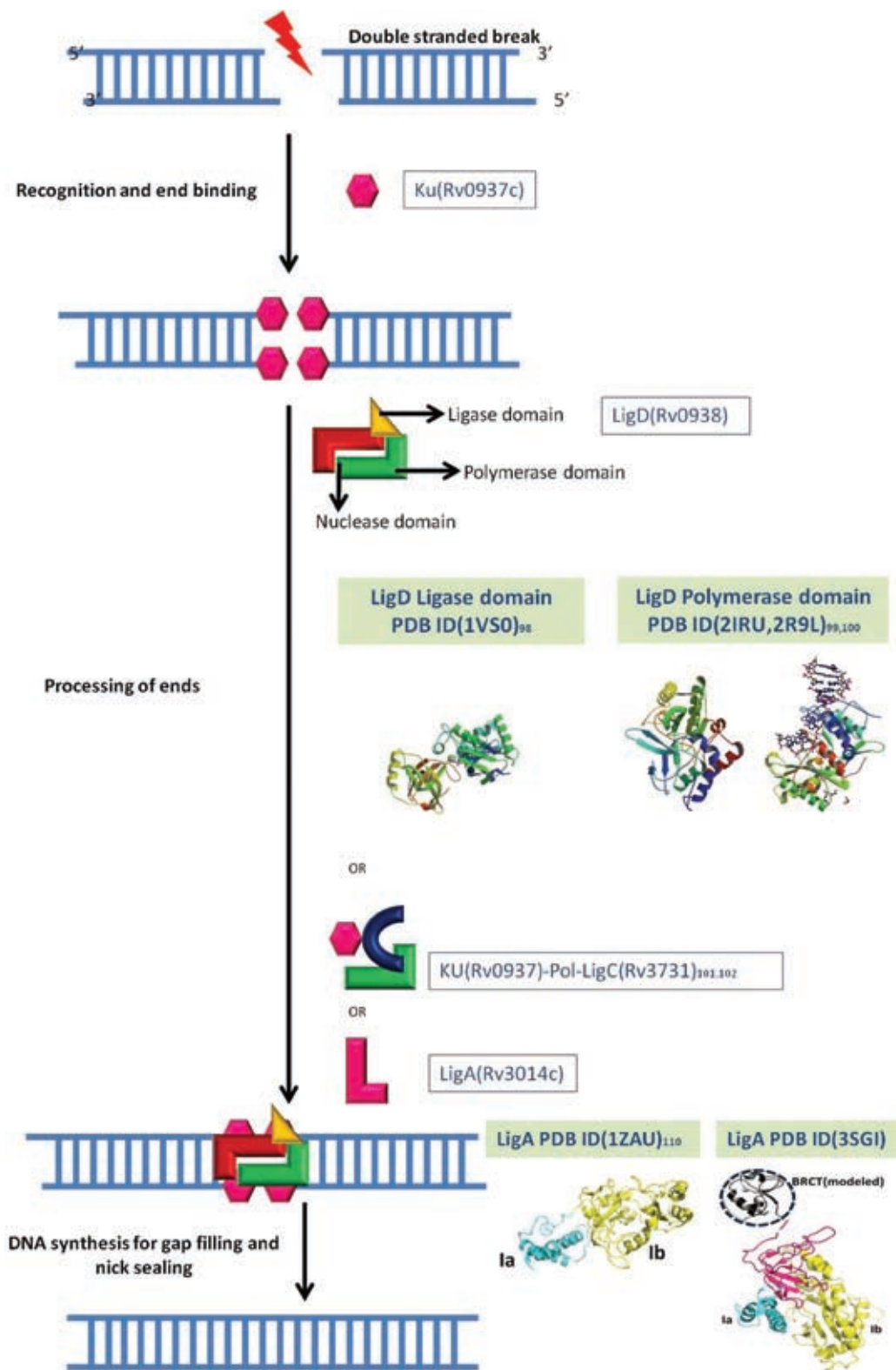


Figure 4: Schematic representation of NHEJ repair pathway in *Mycobacterium tuberculosis*.

be processed into ssDNA by the action of helicases and nucleases.⁸⁸⁻⁹⁰ RecA-dependent pathways include the RecBCD pathway and the RecF pathway. Sequencing of the *M. tuberculosis* genome has

led to the identification of a number of mycobacterial homologs of recombination genes including RecA, RecBCD, RecF, RuvA, RuvB and RuvC.^{49,51} This indicates that the basic mechanism of

recombination is conserved in mycobacteria. The elucidation of crystal structures of mycobacterial RecA and RuvA from have shed more light on the mechanism of these important proteins. The information can be exploited for development of rational design of inhibitors.^{91,92} Recently another heterodimeric helicase–nuclease AdnAB has been identified in mycobacteria. The studies suggest that these play an important role in homologous recombination. They also suggest that mycobacteria are exceptional in that they encode both AdnAB and RecBCD, and furthermore, point to the existence of alternative end-resecting motor–nuclease complexes.⁹³ Analysis of genome sequences identified potential prokaryotic homologs of two proteins known to be involved in NHEJ, namely Ku and an ‘NHEJ-type’ of DNA ligase, *viz.* LigD. NHEJ, was initially assumed to be restricted to eukaryotes till it was identified in mycobacteria. NHEJ is a error prone repair process that proceeds without the need of a homologous DNA molecule, with the direct reattachment of the two broken DNA ends.^{53,94–97} Structural and biochemical characterization of recombinant MtKu and MtLigD suggests they efficiently process NHEJ functions.^{54,97–100} It has been proposed that in the stationary phase, bacteria rely on NHEJ in a manner analogous to non-cycling eukaryotic cells.^{54,97} However, the present understanding supports that the NHEJ process in mycobacteria is more than just a two component system comprising MtKu and MtLigD. Alternate NHEJ processes have been identified in mycobacteria that are independent of Ku and LigD. The latter involves the repair of 3’ overhang Double Strand Breaks and the principal replicative ligase, *viz.* LigA, is involved in this pathway. The role of other as-yet-unknown factors have also to be investigated.^{101,102} There have been speculations about the cross talk between the RecA dependent repair pathways and NHEJ in bacterial systems, known to repair similar kind of DNA damage.¹⁰³ It has been proposed that mycobacteria exploit three genetically distinct DNA double-strand break repair pathways with recent identification of another novel repair system called as ‘Single-strand Annealing’ (SSA) pathway which involves RecBCD complex.¹⁰⁴ The SSA pathway is mutagenic as it involves the loss of genetic material and was initially identified in eukaryotes. Thus, these proteins involved in recombination-based DNA repair proteins, emerge as potential targets for the development of anti-bacterial agents. Most noteworthy are the RecBCD and AddAB helicase-nucleases. Being essential in the DNA repair pathway, their candidature as drug targets is strengthened by its wide distribution

in bacteria and apparent absence from eukaryotes. Also, they contribute to induced mutations, which are known causes of resistance to existing antibiotics. Drugs against these enzymes should expectedly self-limit the evolution of bacterial resistance.

4 DNA Ligases: Novel Drug Targets Waiting for Right Inhibitor

The largest number of new inhibitor classes are presently available for NAD⁺-dependent DNA ligase (LigA), the principal bacterial replicative ligase. DNA ligases are indispensable enzymes playing a critical role in DNA metabolic processes including DNA replication, recombination, and repair in all living organisms. DNA ligases catalyze the joining of nicks between adjacent bases of double-stranded DNA by mediating the formation of phosphodiester bonds at single stranded or double stranded breaks between adjacent 5’ phosphoryl and 3’ hydroxyl groups of DNA. The first step consists of the formation of a covalent DNA ligase-adenylate intermediate. In the second step, AMP is transferred from DNA ligase to the 5’phosphate of nicked DNA through a pyrophosphate bond. In the third step, a phosphodiester bond is formed to join adjacent polynucleotides, with the release of AMP.¹⁰⁵ DNA ligases can be classified into two groups on the basis of their specificities of cofactor used for the formation of DNA ligase-adenylate intermediate: NAD⁺-dependent DNA ligases are present in bacteria, some entomopox viruses and mimi virus while ATP dependent DNA ligases are ubiquitous.^{106–108} Mycobacteria contain genes for several ATP-dependent DNA ligases and a single NAD⁺-dependent DNA ligase encoded by *ligA*.^{109–112} LigA has recently drawn attention as a broad-spectrum novel antibacterial target because it is essential for DNA replication, conserved among bacterial pathogens, and markedly dissimilar to eukaryotic DNA ligases. It has particularly drawn attention in case of mycobacterium as a target with the potential to combat multiple drug resistance.^{110,113–117} The recent determination of X-ray crystal structures of several DNA ligases has provided insights into substrate binding and the catalytic mechanisms. The information gained from structural studies has motivated rational inhibitor design and identification also with the utilization of information gained from of a broad range of techniques encompassing molecular biology, protein biochemistry and synthetic chemistry. Most of the present crop of inhibitors bind to the co-factor binding site, and efforts have involved the design of inhibitors that can distinguish between NAD⁺ and

ATP-dependent ligases respectively. In this respect, a spectrum of inhibitors have been identified that includes alkaloids from the simple quinoline, isoquinoline berberine, quinacrine, benzophenanthridine alkaloids, flavonoids, flavonoxanthone, triterpenes, bisquinolines, anthracycline analogs, and Pyridochromanones.^{114,116,118–133} Our group, in the first instance, has searched for diverse compound families which inhibit MtbLigA with several fold specificity compared to ATP-dependent ligases (human DNA ligase I). We have identified glycosyl ureides, glycofuranosylated diamines, tetracyclic indole derivatives, dispiro-cycloalkanones and hydroxamates as novel inhibitors possessing the potential to be developed as promising antibacterials against *M. tuberculosis*. These compounds possess IC_{50} values in the low μ M range and compete with NAD^+ .^{111,113,134} Given the conserved nature of the cofactor binding site, most of the inhibitors are expected to exhibit some degree of general antibacterial activity too. Better inhibitor development is focused on improving the specificity of the compounds for MtbLigA. The major approaches in the direction of achieving better inhibitor development is by utilizing active site water molecules.¹³⁵ Other ongoing approaches involve the design and synthesis of inhibitors that can bind to other regions of the molecule, eg. the BRCT domain. The latter inhibitors will expectedly block subsequent catalytic steps.¹³⁶ We have demonstrated that the BRCT domain of MtbLigA is important for bacterial survival.¹¹¹ The BRCT domain has been shown to play an important role in mediating protein-protein interactions in other homologous enzymes. Compounds designed to bind to BRCT domain to disrupt its interactions, eg. those that disrupt the interactions of important conserved residues like a conserved glycine in MtbLigA¹³⁷ should have different modes of action compared to the earlier LigA inhibitors. As a start, we modeled the MtbLigA BRCT domain and suggested that it possesses regions with finer structural differences. The studies¹³⁶ pave the way for a full-blown exploration of inhibitors with this mode of action.

5 Concluding Notes for the Future, Especially in Tuberculosis Treatment

There is increased international attention on tuberculosis in the last decade that is in part attributable to whole genome sequencing of *M. tuberculosis* and other mycobacterial variants and strains. Structural genomics approaches have also resulted in the spurring of rational design strategies for new inhibitor design. Presently, there are at least 8 compounds or combinations

in Phase II clinical trials and 4 compounds in Phase III trials (<http://www.newtbdrugs.org/pipe-line.php>) against tuberculosis. A brief analysis of those in Phase III trials is in order. Rifamycin, one of the compounds, is thought to inhibit DNA-dependent RNA polymerase. Resistance issues to the compound have been observed and have been attributed to missense mutations in the rpoB gene. Delamanid, an oxazole derivative is another compound undergoing trials and was being examined for its efficacy against multi-drug resistant TB. However, the committee for medicinal products for human use (CHMP) of the European Medicines Agency has very recently voted not to recommend marketing authorization for Delamanid as its supposed benefits have not been sufficiently demonstrated. Gatifloxacin, a quinolone antibiotic introduced in 1999, which is a DNA gyrase/topoisomerase IV inhibitor, is also undergoing trials as a pulmonary TB therapeutic. It is worth noting that several earlier reports have established severe side effects for Gatifloxacin including that of developing severe hyperglycemia. In fact it was banned in India in 2011 for systemic use. Moxifloxacin, another relatively old quinolone antibiotic, is also undergoing trials. However, its oral use is approved with the warning that it may cause worsening of symptoms for those with Myasthenia gravis, a disease associated with muscle weakness and breathing problems. It is clear that most of the compounds undergoing Phase III trials have several problems or are antibiotics that are being repositioned for TB treatment. Delamanid, the relatively new experimental drug has severe problems; however a related molecule, PA-824, a Nitroimidazole-oxazine, is showing promise in Phase II trials of being developed as a new therapeutic. The latter has a complex novel mechanism of action and is active against both replicating and non-replicating TB. PA-824 is a prodrug that requires reductive activation of an aromatic nitro group that is carried out by glucose-6-phosphate dehydrogenase, Rv0407.¹³⁸ TMC207, a diarylquinoline, also called bedaquiline, is another promising new drug that acts by inhibiting the mycobacterial ATP synthase. It is also active against drug-resistant TB. Long-term safety is yet to be tested fully for this moiety and Phase III trials are presently under development. However, TMC207 holds out the exciting promise of reducing treatment duration by half, as suggested by the preliminary data. It is clear that upcoming therapeutics are important to face current and future TB treatment challenges. They are mainly dependent on the addition of a single new drug to an already failing (against drug-resistant TB) treatment regimen or involve

repositioning old drugs. Based on past history and the devious means adopted by the pathogen, there is an urgent need to keep ahead of its resistance mechanisms. It is hoped that sustained global interest in the eradication of TB as a universal health crisis will finally succeed in the not-too-distant future.

In this context, targeting the DNA repair and metabolism processes of bacteria seems to be an attractive strategy for the development of therapeutics that are expectedly self-limiting from the point of view of developing resistance, both in mycobacteria and other drug-resistant bacteria.

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