Double-Strand DNA Break Repair in Mycobacteria

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ABSTRACT Discontinuity of both strands of the chromosome is a lethal event in all living organisms because it compromises chromosome replication. As such, a diversity of DNA repair systems has evolved to repair double-strand DNA breaks (DSBs). In part, this diversity of DSB repair systems has evolved to repair breaks that arise in diverse physiologic circumstances or sequence contexts, including cellular states of nonreplication or breaks that arise between repeats. Mycobacteria elaborate a set of three genetically distinct DNA repair pathways: homologous recombination, nonhomologous end joining, and single-strand annealing. As such, mycobacterial DSB repair diverges substantially from the standard model of prokaryotic DSB repair and represents an attractive new model system. In addition, the presence in mycobacteria of a DSB repair system that can repair DSBs in nonreplicating cells (nonhomologous end joining) or when DSBs arise between repeats (single-strand annealing) has clear potential relevance to *Mycobacterium tuberculosis* pathogenesis, although the exact role of these systems in *M. tuberculosis* pathogenesis is still being elucidated. In this article we will review the genetics of mycobacterial DSB repair systems, focusing on recent insights.

Repair of double-strand DNA breaks (DSBs) is critical to all living organisms. Scission of the phosphodiester backbone of both DNA strands is lethal if not repaired because such loss of linear chromosome integrity compromises chromosome replication and thereby prevents genome duplication. In contrast to some other types of DNA lesions which can be bypassed by damage-tolerant DNA polymerases, there is no known mechanism for the replication or transcription machinery to bypass a DSB, mandating their repair before replication or transcription can proceed. As such, multiple systems have evolved to repair DSBs, from bacterial to human cells (1–6). In the past decade, mycobacterial DNA repair systems in general, and mycobacterial DSB repair systems in particular, have received increasing attention. It has become clear that mycobacterial DSB repair differs substantially from the standard models of prokaryotic DSB repair derived from work in the *Escherichia coli* system. Most prominent among these differences is the existence of two additional DSB repair pathways that are not present in *E. coli* and were previously thought not to exist in bacteria: nonhomologous end joining (NHEJ) and single-strand annealing (SSA). Multiple other novel features of mycobacterial DSB repair have also been elucidated, making mycobacteria a new model system for the study of prokaryotic DSB repair. As now conceptualized, mycobacterial DSB repair actually most resembles DSB repair in budding yeast rather than other prokaryotes (Table 1). In addition to its emerging place as a model system, studies of mycobacterial DNA repair also are of great importance for understanding mechanisms of mutagenesis and genome diversification in *Mycobacterium tuberculosis*, the ultimate cause of antimicrobial resistance in *M. tuberculosis* (7). In addition to the information and references contained in this article, the reader is pointed to several excellent recently published reviews of mycobacterial DNA repair and mutagenesis (8–10).
Although DSBs are induced experimentally by ionizing radiation or clastogenic chemicals such as bleomycin, the doses of ionizing radiation used in laboratory experiments are not often encountered in nature. The major sources of physiologic DSBs that arise in vivo are likely to be (i) DNA replication across a single-strand nick, (ii) oxidative damage, (iii) desiccation, and potentially, (iv) ribonucleotides incorporated into DNA (11, 12). The frequency and number of DSBs that arise in a mycobacterial cell are difficult to estimate, and in particular, it is difficult to know the frequency and character of DNA lesions that *M. tuberculosis* experiences within its human host. We will review the three pathways of DSB repair in mycobacteria, with emphasis on recent genetic studies.

**HOMOLOGOUS RECOMBINATION**

Homologous recombination (HR) is a universally distributed mechanism of DSB repair throughout all domains of life. From bacterial to human cells, HR proceeds through a common set of biochemical transactions that share common themes. By definition, homology-directed repair of a DSB requires an unbroken homologous chromosome to direct repair and therefore must occur after chromosome replication has occurred. The DNA structure ultimately used for homology search and strand invasion into the homologous duplex is a 3′ single-stranded DNA coated with a strand invasion protein, RecA, in bacteria. Therefore, the initiating step in HR is recognition of the break by proteins that initiate resection to generate this 3′ single-stranded substrate. In *E. coli*, this resection is accomplished by the RecBCD enzyme complex (2, 13). RecBCD ressects double-stranded DNA from the DSB until it encounters a Chi site, an 8-bp recognition sequence distributed throughout the *E. coli* chromosome. Upon encountering Chi, RecBCD converts to a single-stranded 5′-to-3′ exonuclease, thereby generating a 3′ single-stranded DNA that is coated by the single-strand binding protein SSB. Exchange of SSB for RecA to form the RecA nucleoprotein filament is facilitated by RecBCD (2).

**TABLE 1** Comparison of DSB repair systems in bacteria and yeast

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Function</th>
<th><em>E. coli</em></th>
<th><em>B. subtilis</em></th>
<th><em>M. smegmatis</em></th>
<th>Saccharomyces cerevisiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>End resection</td>
<td>RecBCD</td>
<td>AddAB</td>
<td>AdnAB + ?</td>
<td>MRX/Sae2/Dna2/Exo1</td>
</tr>
<tr>
<td></td>
<td>Single-strand protection</td>
<td>SSB</td>
<td>SSB1 (SSB2)</td>
<td>SSBI (SSB2)</td>
<td>Rpa</td>
</tr>
<tr>
<td></td>
<td>Mediator (RecA loading)</td>
<td>RecBCD (RecFOR)</td>
<td>RecFOR (?)</td>
<td>RecFOR (?)</td>
<td>Rad52</td>
</tr>
<tr>
<td></td>
<td>Strand exchange</td>
<td>RecA</td>
<td>RecA</td>
<td>RecA</td>
<td>Rad51</td>
</tr>
<tr>
<td>NHEJ</td>
<td>End binding</td>
<td>Ku (ykoV)</td>
<td>Ku</td>
<td>Ku</td>
<td>Ku70/Ku80</td>
</tr>
<tr>
<td></td>
<td>End sealing</td>
<td>LigD (ykoU)</td>
<td>LigD-Lig (LigC)</td>
<td>LigD-Lig (LigC)</td>
<td>Lig4</td>
</tr>
<tr>
<td></td>
<td>End remodeling: nucleotide addition</td>
<td>? (No PE domain in BSuLigD)</td>
<td>LigD-PE</td>
<td>LigD-PE</td>
<td>Tpp1</td>
</tr>
<tr>
<td></td>
<td>End remodeling: phosphatase</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>End remodeling: nuclease</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>?</td>
</tr>
<tr>
<td>SSA</td>
<td>End resection</td>
<td>Pathway not described</td>
<td>Pathway not described</td>
<td>RecBCD</td>
<td>Exol/MRX/SgsI/Dna2</td>
</tr>
<tr>
<td>Strand annealing</td>
<td>RecO</td>
<td>Rad52</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Major factors for each pathway are listed with secondary factors listed in parentheses. Please see text for details.*
ΔadnAB strain in clastogen sensitivity, a finding inconsistent with redundancy between these two enzyme complexes (14). Although these studies provided some insight into the epistatic relationships between recBCD and adnAB, clastogen sensitivity assays invariably involve multiple types of DNA damage and therefore do not specifically interrogate the function of a particular factor in repair of specific DNA lesions.

Clarification of the roles of mycobacterial AdnAB and RecBCD in recombination came from studies using a chromosomal reporter system in which a single chromosomal break is induced by the homing endonuclease I-SceI. In this system, two 18-bp recognition sites for I-SceI were placed into a defective lacZ allele with a downstream LacZ donor segment. A key feature of this system is that one of the two I-SceI sites is inverted such that I-SceI cleavage at both sites creates a DSB with incompatible ends. This break cannot be directly ligated by NHEJ without end modification. This configuration seeks to minimize repeated cycles of I-SceI cutting and resealing that can occur with a single I-SceI site and which obscure conclusions about the frequency of each repair pathway. Chromosomal breakage is induced by transfection with an I-SceI encoding plasmid. Three repair outcomes are possible in this system, all of which can be distinguished by scoring the surviving colonies on X-Gal- and kanamycin-containing media (14): NHEJ, HR, and SSA (Fig. 1). When this assay is performed in strains lacking putative repair factors, the role of each can be deduced by examining the repair outcomes in this genetic background.

When repair outcomes were assayed using the I-SceI system in strains lacking recBCD, adnAB, or both, surprising findings emerged. First, RecBCD was not required for HR in mycobacteria, because the percentage of HR outcomes in the I-SceI system was actually increased above wild type in ΔrecBCD M. smegmatis (14). Deletion of adnAB resulted in an approximately 50% decrement in HR outcomes, supporting a role for adnAB in DSB resection in vivo. Notably, some residual HR events, which were all recA dependent, were preserved in the ΔrecBCD ΔadnAB strain, indicating an additional

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**FIGURE 1** Pathways of DSB repair in mycobacteria. Our present understanding of DSB repair in mycobacteria. The three pathways shown are HR, NHEJ, and SSA. For each pathway, the major DNA processing events are depicted with the factors required for each step, when known. A question mark indicates that no specific experimental genetic data is available about that step, despite the presence of predicted proteins in mycobacterial chromosomes that may mediate these steps, or even biochemical activities consistent with a role in these pathways. In the NHEJ column, the three outcomes below the arrow indicate faithful repair, nucleotide addition, and nucleotide trimming, respectively. In the SSA column, the blue rectangles indicate repeat sequences that flank the DSB. Please see text for further details and references. doi:10.1128/microbiolspec.MGM2-0024-2013.f1
pathway of DSB resection that is yet to be defined (14). Additional conclusions about the function of RecBCD in mycobacteria were gleaned from these experiments, which are discussed below in the section about the SSA pathway.

**MEDIATOR FUNCTION IN MYCOBACTERIAL RECOMBINATION**

After resection of the DSB to create the 3’ single-stranded tail, the single strand is coated by SSB. SSB has a very high affinity to single-stranded DNA, so loading of RecA onto the 3’ single strand requires exchange of SSB for RecA to create the RecA nucleoprotein filament, which is the agent of homology search and strand invasion. This exchange reaction is facilitated by a mediator complex, a function that is conserved in all domains of life. In *E. coli*, mediator function is supplied by RecBCD, which has a RecA loading function. An additional mediator function is supplied by the RecFOR complex, which is evident in *E. coli* when RecBCD is inactivated but is a more prominent mediator in wild-type *B. subtilis* cells (17). In yeast, the major mediator is Rad52, which participates in Rad51 loading; in human cells, BRCA2 is the major mediator (Table 1).

Mediator function in mycobacteria has not been investigated in depth. The finding that RecBCD in mycobacteria does not participate in RecA-dependent HR would seem to exclude a mediator function for RecBCD. AdnAB, as a resection nuclease in mycobacterial HR, is a candidate to have RecA loading function, analogous to the mediator function of RecBCD in *E. coli*, but this function has not yet been demonstrated. A recent publication examined the function of the RecO protein in mycobacteria. RecO is a widely conserved bacterial protein that is the central component of the RecFOR complex (18). *M. smegmatis* lacking *recO* was found to be viable but highly sensitive to a variety of clastogens, including ionizing radiation. Importantly, the ΔrecO strain was nearly as sensitive as the ΔrecA strain, and more sensitive than the ΔadnAB strain, suggesting a major role for the RecFOR system in mycobacterial recombination (18).

The phenotype of the *M. smegmatis* ΔrecO strain in the I-SceI recombination system confirmed a major role in HR. HR events in the ΔrecO strain were significantly reduced compared to the wild-type strain, although some residual HR was present (18). Examination of the ΔrecOΔadnAB strain in this assay revealed a complete lack of HR events, indicating that RecO and AdnAB define two parallel pathways that lead to RecA-dependent recombination in mycobacteria. These results leave several questions unanswered. Although the results clearly support two parallel pathways of end resection and mediator function in RecA-dependent HR, multiple components remain to be identified.

In the RecO pathway, the identity of the resection nuclease is unknown. In the RecFOR system of *E. coli*, the nuclease that resects the DSB is RecJ, but no RecJ protein has been identified in mycobacteria. In addition, the functions of the RecF and RecR proteins in mycobacterial HR have not yet been examined. In the AdnAB pathway, the mediator for RecA loading after AdnAB-mediated resection is not known and will be the subject of future experimentation.

**OTHER RECOMBINATION FACTORS**

Unfortunately, the focus of this article on genetic studies of DSB repair does not permit a full discussion of extensive studies that have been performed on recombination proteins from mycobacterium and the regulation of their expression. These include the biochemical feature of RecA (19–22), the regulation of the DNA damage response in mycobacteria (23–27), and studies on Holliday junction resolvases (28–31). The reader is referred to these articles for in-depth discussion of these important topics.

**NHEJ IN BACTERIA**

**Historical Perspective**

The standard model of prokaryotic double-strand DNA break repair was derived from seminal studies in *E. coli* that defined, over many decades, the molecular requirements for HR. These studies established the primacy of RecA as the dominant strand exchange protein in bacterial recombination. However, the existence of alternative DSB repair pathways in *E. coli* was not documented, and such pathways were presumed not to exist. This situation is in stark contrast to the plethora of DSB repair pathways that have been intensely studied in eukaryotic cells and which include HR, NHEJ, and SSA. As genomic sequencing of broad bacterial phyla revealed new prokaryotic protein families, bioinformatic analysis of these genomes revealed clues to a greater diversity of DSB repair pathways than had been deduced from studies in *E. coli*. Foremost among these observations was the finding that some bacterial genomes, including those of *M. tuberculosis* and *M. smegmatis*, encoded apparent orthologs of the Ku protein (32). In eukaryotic organisms, the Ku70/Ku80
heterodimer binds DNA ends and acts as an organizing hub for NHEJ. The existence of Ku in bacteria suggested the existence of an NHEJ pathway. Tantalizing additional bioinformatic observations identified ATP-dependent DNA ligases, sometimes in close genomic proximity to the gene encoding Ku, in these bacteria. The conserved bacterial DNA ligase, LigA (33), is an NAD-dependent enzyme, whereas eukaryotic ligases, such DNA ligase IV, which mediates NHEJ, are ATP dependent.

**In Vivo Evidence for a Bacterial NHEJ Pathway**

Early biochemical studies of the putative NHEJ proteins *M. tuberculosis* LigD and Ku demonstrated that these proteins can cooperate to seal DNA ends in vitro (34, 35). Definitive evidence of the existence of an NHEJ pathway in vivo initially came from studies using transfection of linear plasmids into *M. tuberculosis* and *M. smegmatis* and mutant strains lacking the genes encoding LigD and Ku. In contrast to *E. coli*, *M. tuberculosis* and *M. smegmatis* can recircularize linear plasmid DNA with either blunt or cohesive ends. For 5' and blunt-end linear plasmids, this recircularization is reduced by approximately 500-fold in strains with null mutations in Ku or LigD (36–38), indicating an active NHEJ pathway that depends on Ku and LigD, which physically interact. The linear plasmid transformation assay is useful for interrogating NHEJ, but because the DNA sequence flanking the plasmid DSB does not have homology to the chromosome, HR-mediated repair is not possible. Therefore, the plasmid assay does not interrogate the relative frequency of NHEJ when alternative repair pathways are possible. Additional evidence for the existence and characteristics of the mycobacterial NHEJ pathway came from the I-SceI system. In wild-type cells carrying the I-SceI recombination substrate in which all repair outcomes are possible, NHEJ-mediated repair constitutes 20% of the repair outcomes (14). These NHEJ events are abolished in strains lacking Ku or LigD, and loss of NHEJ leads to a compensatory increase in HR (14).

As noted above, NHEJ in bacteria was first demonstrated using linear plasmid transfection and homing endonuclease-induced DSBs. In yeast and human cells, NHEJ is critical for DSB repair when a chromosomal homolog is not available to provide a template for strand invasion (3). By analogy, NHEJ in bacteria should be critical for clastogen resistance in stationary phase or other states in which chromosome replication has ceased and/or the cell is monochromosomal. Spores of *B. subtilis* are monochromosomal and nonreplicating and thereby provide a physiologic state in which NHEJ should be important for genomic integrity. Accordingly, Ku-deficient *B. subtilis* spores are highly sensitive to dry heat or hydrogen peroxide (in an α/β small acid-soluble spore protein [SASP] mutant background; 39). Similarly Ku- or LigD-deficient *M. smegmatis* in late stationary phase is sensitive to ionizing radiation, a sensitivity that is not observed in log-phase cells or in stationary-phase cells grown in rich media (15). Additional studies have also shown that NHEJ protects stationary-phase mycobacteria from desiccation (40).

In summary, *M. tuberculosis* and *M. smegmatis* encode a pathway of NHEJ that repairs DSBs in late stationary-phase cells and is dependent upon Ku and LigD. These experiments indicated that these two proteins, Ku and LigD, perform many (although not all; see below) of the repair functions of NHEJ. In contrast, eukaryotic NHEJ has multiple additional proteins that participate in end remodeling during NHEJ, including polymerases and nucleases that remodel the broken ends into ligatable termini (3). In the mycobacterial NHEJ system, some of these end remodeling functions are supplied by autonomous enzymatic functions encoded with the LigD protein.

**Biochemical Functions of the LigD Protein and Their Role in NHEJ In Vivo**

The LigD protein from *M. smegmatis* and *M. tuberculosis* has three identifiable enzymatic domains, polymerase (POL), phosphoesterase (PE), and ligase (LIG). Detailed biochemical, structural, and genetic studies have elucidated the NHEJ functions of each of these domains.

**LigD-LIG**

The LigD-LIG domain is an ATP-dependent DNA ligase with a classic three-step ligase mechanism (38, 41). As noted above, deletion of the ligD gene from *M. tuberculosis* or *M. smegmatis* reduces the efficiency of NHEJ for both plasmid and chromosomal substrates. Surprisingly, ablation of the LigD-LIG active site in vivo (through replacement of the active site lysine with alanine) surprisingly did not phenocopy the severe NHEJ defect observed in the ΔligD strain (36, 41). NHEJ efficiency was substantially preserved, although these LigD-LIG-independent NHEJ events were low fidelity (36). This result indicated that another DNA ligase can supply strand sealing activity when LigD-LIG is inactivated, and further studies indicated that this activity is likely supplied by Ligase C (36).
LigD-POL
A remarkable feature of mycobacterial NHEJ is the frequent addition of template and nontemplated nucleotides to the repaired ends (36, 37). These end modifications suggest the participation of a polymerase in mycobacterial NHEJ. The POL domain of LigD is a primase-like polymerase that can add both templated and nontemplated nucleotides to DNA in vitro (37, 42, 43). LigD-POL is also proficient at adding ribonucleotides, a property it shares with polymerases that participate in eukaryotic NHEJ (44). Ablation of the LigD-POL domain activity by mutation of the di-aspartate metal binding site abolishes mutagenic NHEJ at blunt ends during plasmid NHEJ but has relatively little effect on the templated fill-in events observed at resealed 5′ overhang plasmid termini (36, 37, 43). The necessity of the LigD protein for NHEJ, which is independent of its ligase activity, is supplied by the POL domain, which, in addition to catalyzing NHEJ additions, also appears to play a structural role in the NHEJ complex (36, 45).

LigD-PE
The LigD-PE domain is the third enzymatic module of the LigD protein that participates in end remodeling. The PE domain from Pseudomonas LigD has been most extensively characterized in vitro. LigD-PE is the founding member of a novel 3′ end healing enzyme family present in bacteria, archaea, and fungi. PE resects the ribonucleotide tract on an RNA-DNA hybrid until a single ribonucleotide with a 3′ OH group remains (46–49). This ribonucleotide-terminated strand is the preferred substrate for the LigD ligase, indicating that the PE and POL domains may cooperate to produce a ribonucleotide-terminated DNA strand that is the ultimate substrate for LigD ligase activity. The role of the PE domain in NHEJ in vivo is less well understood because it does not participate in the efficiency or fidelity of plasmid NHEJ (36).

Additional Potential NHEJ Factors
Several additional factors have been identified in mycobacteria that may participate in NHEJ. As noted above, the LigD-POL domain is a primase-like polymerase that is the direct catalyst of nontemplated mutagenic NHEJ in vivo. The persistence of templated fill-in events when LigD-POL activity is inactivated indicated the participation of additional polymerases in mycobacterial NHEJ. Prime candidates for this activity were the PolD1 and PolD2 enzymes. PolD1 and PolD2 are freestanding LigD-POL like enzymes without the Lig and PE domains contained in the LigD polypeptide. PolD1 and PolD2 have biochemical activities similar to LigD-POL, including a preference for ribonucleotide addition over deoxyribonucleotides and both template and nontemplated polymerase activity (50). Despite the suspicion that PolD1 and PolD2 participate in NHEJ based on their activities and genomic proximity (in the case of PolD1) to the backup NHEJ ligase LigC, ablation of PolD1 and PolD2 in combination in vivo did not affect NHEJ fidelity of 5′ overhangs, even when LigD-POL was inactivated (50). These results indicate that there are as yet unidentified polymerases that participate in NHEJ, which may be redundant with LigD-POL, PolD1, and PolD2.

Experiments seeking mycobacterial proteins interacting with Ku identified UvrD1 as a Ku interacting protein (51). Ku stimulates UvrD1 helicase activity in vitro, and ablation of UvrD1 sensitizes M. smegmatis to ionizing radiation in all growth phases (51), but deletion of UvrD1 does not impair NHEJ-mediated resealing of linear plasmids. The exact function of the Ku-UvrD1 interaction in mycobacterial DSB repair remains to be elucidated. Additional efforts to identify Ku interacting proteins identified the protein encoded by Msmeg_5175, which has homology to NAD-dependent deacetylases of the Sir2 family. Deletion of Msmeg_5175 in M. smegmatis reduced NHEJ efficiency and conferred sensitivity to ionizing radiation, although not to the degree of the M. smegmatis Δku strain (52).

SSA PATHWAY
Single-strand annealing (SSA) is a mechanism of DSB repair that occurs when the DSB is flanked by repeats. Bidirectional single-strand resection from the break reveals complementary single strands that can anneal. Subsequent flap removal, fill-in synthesis, and ligation result in repair of the break with a deletion and single remaining repeat (Fig. 1). SSA has been described in yeast and is independent of the eukaryal stand exchange protein Rad51 but dependent on the Rad52 protein (53). However, SSA was not described as a DSB repair pathway in bacteria. Use of the I-SceI recombination system described above allowed detection of recombination events consistent with an SSA mechanism of repair. Specifically, approximately 15% of repair events at an I-SceI-induced DSB resulted in a deletion of the intervening DNA between two lacZ repeats. Importantly, these repair events were independent of RecA, consistent with an SSA mechanism of repair (14). Surprisingly, although deletion of RecBCD did not reduce HR events (see above), SSA...
events were abolished in a RecBCD null strain (14). In conjunction with the lack of any clastogen sensitivity of the RecBCD strain, this finding strongly indicated that RecBCD in mycobacteria has a function distinct from its well-established role as a resection nuclease and RecA mediator in the E. coli HR pathway.

Additional studies with an M. smegmatis strain lacking RecO indicated a substantial role for RecO in the mycobacterial SSA pathway. A deletion mutant of M. smegmatis lacking RecO was severely compromised in executing SSA compared to wild-type cells (18). Consistent with this involvement in the SSA pathway, purified RecO protein displayed a zinc-dependent DNA binding activity and accelerated the annealing of single-stranded DNA (18).

As a pathway that repairs DSBs that arise between repeats, the SSA pathway may have particular relevance to M. tuberculosis genome evolution. The M. tuberculosis chromosome contains repetitive DNA in the form of PE, PPE (Pro-Pro-Glu), and PGRS (polymorphic GC-rich sequence) elements, which account for a substantial fraction of the M. tuberculosis chromosome. DSBs that may arise within these regions are prime candidates to be repaired by the SSA pathway (or NHEJ for that matter) but are problematic for repair by HR because of difficulty in finding the appropriate homologous sequence for RecA-dependent strand invasion. Whether SSA-mediated repair would preferentially operate within repetitive regions of the mycobacterial chromosome remains to be tested, but it is potentially relevant that studies of the M. tuberculosis genome variation have found that PE, PPE, and PGRS genes do contain sequence polymorphisms that could be consistent with either SSA- or NHEJ-mediated repair (54–57), and studies of instability of direct and inverted repeats in mycobacteria have implicated these pathways (58).

ROLE OF DNA REPAIR IN M. TUBERCULOSIS PATHOGENESIS

It is clear that the products of the mammalian immune system can damage DNA through base damage, sugar damage, or phosphodiester strand breakage. It is also clear that several bacterial pathogens rely on DNA repair systems to resist the clastogenic environment of the host, including Helicobacter pylori, Salmonella enterica, and Neisseria meningitidis (see references in reference 8). However, despite the strong suspicion that M. tuberculosis uses DNA repair pathways to survive in vivo, demonstration of the importance of repair pathways in animal models of infection has been difficult. As has been suggested (8), the lack of dramatic pathogenesis phenotypes of some DNA repair mutant strains may reflect redundancy among repair mechanisms or reflect the relative gross nature of bacterial load assays to detect significant changes in bacterial chromosome structure that may occur independently of net bacterial survival. Nevertheless, some important findings have implicated specific DNA repair pathways in M. tuberculosis pathogenesis and in vivo mutagenesis.

Several studies have examined the role of nucleotide excision repair (NER) in M. tuberculosis pathogenesis. After its identification as a factor conferring resistance to nitric oxide (59), an M. tuberculosis ΔuvrB strain was tested in the mouse model of infection and found to be mildly attenuated by CFU and time to death assays (60). The attenuation phenotype of the ΔuvrB strain was reversed in phox/iNOS−/− mice, indicating that the NER system does defend against host immune pressure in vivo. A recent study examined the role of the UvrD1 helicase in M. tuberculosis pathogenesis (61). UvrD is a component of the NER machinery and in this function removes the incised oligonucleotide after strand cleavage by UvrABC. However, UvrD also has additional functions in recombinination, and in mycobacteria there are two UvrD-like proteins, UvrD1 and UvrD2. As noted above, UvrD1 interacts directly with Ku, whereas UvrD2 is essential (62, 63), although this essential function is not supplied by its helicase activity (63).

The Davis group examined ΔuvrA, ΔuvrD1, and ΔuvrAΔuvrD1 strains in the mouse model of infection. The ΔuvrA strain was mildly attenuated, comparable to previously reported findings with the ΔuvrB strain. The ΔuvrD1 strain was attenuated by ∼1.5 logs at 50 days postinfection and by ∼3 logs at 150 days. Dramatically and somewhat surprisingly, the ΔuvrAΔuvrD1 strain was more attenuated than either single mutant and barely replicated in mice, a phenotype that was complemented by uvrD1 (61). The attenuation phenotype of this double mutant is the most dramatic attenuation phenotype reported for a DNA repair–deficient strain of M. tuberculosis and clearly indicates the value of combining mutations from distinct repair pathways (in this case presumably reflecting inactivation of NER through loss of uvrA and the non-NER functions of UvrD1).

Another dramatic example of the effect of a DNA repair pathway on M. tuberculosis infection and in vivo mutagenesis came from the examination of the dnaE2 mutant by the Mizrahi group. Inactivation of dnaE2 conferred attenuation to M. tuberculosis in mice as measured by CFU and time to death analyses (64). In
addition, the ΔdnaE2 strain failed to evolve resistance to rifampin, providing a clear example that an error-prone repair pathway is essential to the evolution of drug resistance in vivo (64).

Despite the substantial advances in understanding the pathways of double-strand DNA break repair in mycobacteria, including the discovery of the NHEJ and SSA pathways as detailed above, the role of DSB repair in *M. tuberculosis* pathogenesis has been difficult to document. Early studies tested a BCG strain lacking recA and found no attenuation in either BALB/c mice or nude mice with intravenous infection (65). As noted above, mycobacteria elaborate three pathways of DSB repair: HR, NHEJ, and SSA. Two of these pathways, NHEJ and SSA, do not require a replicated chromosome to direct repair and therefore may be relevant to stages of *M. tuberculosis* infection during which replication has ceased. In addition, the presence of three repair pathways may suggest redundancy that would mask the phenotypes of *M. tuberculosis* strains lacking a single DSB repair pathway. Extensive experimentation in the Glickman lab has examined the pathogenesis phenotypes of *M. tuberculosis* Δ*Δku, ΔrecA*, and Δ*Δku ΔrecA* in multiple mouse models of infection including C57BL/6 and C3HHeB/Fe mice (66) and in guinea pigs. Surprisingly, these experiments did not demonstrate any change in bacterial load in any of these mutant strains, in any of the animal models, compared to wild-type *M. tuberculosis* (M. Glickman, in preparation).

These surprising findings fail to document a role for DSB repair in *M. tuberculosis* pathogenesis. The lack of phenotype of NHEJ-deficient strains in the mouse model could be attributed to the lack of true growth arrest/latency in these models (67), although the lack of phenotype in the recA ku double mutant cannot be explained by ongoing replication. The possibility exists that the murine immune response to *M. tuberculosis* is not capable of inflicting DSB-inducing genotoxic stress on the *M. tuberculosis* chromosome. The lack of phenotype of these DSB repair–deficient strains fits with other studies that show a similar lack of phenotype for DNA repair–deficient strains (68) and, as has been suggested by others, may reflect the relative gross measure of bacterial load as an outcome (8). More granular data about the role of individual repair pathways in pathogenesis may come from examination of chromosomal mutagenesis and rearrangements by next generation sequencing of DNA repair pathway mutant strains passaged through animal models (69).

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Double-Strand DNA Break Repair in Mycobacteria


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