Synopsis

Physiological importance of DNA repair in mycobacteria

Mycobacteria represent an important group of human pathogens. The members of this group are characterized by high G+C content in the genome and consequently are at a greater risk of deamination of cytosine and oxidation of guanine leading to formation of uracil and 8-oxo guanine, respectively. Inability to repair these modified bases leads to accumulation of mutations that threaten the survival of bacteria. Genome analysis reveals the lack of mismatch repair homologues in mycobacteria implicating a greater importance of the base excision and the nucleotide excision repair (NER) systems. Although the DNA repair mechanisms in mycobacteria are not well understood, these are vital for the pathogen's subsistence in the host macrophages. In this study, we have compared the role of different DNA repair systems using targeted mutant strains that lack uracil DNA glycosylase (Ung), formamidopyrimidine DNA glycosylase (Fpg or MutM) or UvrB (NER) in a fast growing non-pathogenic member of mycobacterium, *M. smegmatis.*

I) Important role of nucleotide excision repair pathway in *Mycobacterium smegmatis* in conferring protection against commonly encountered DNA damaging agents.

Our studies show that the strain deficient in NER is the most sensitive to commonly encountered DNA damaging agents such as UV, low pH, reactive nitrogen intermediates (RNI), reactive oxygen species (ROS) and DNA damage inducible environments such as intracellular environment of macrophages whereas the loss of Ung leads to a higher spontaneous mutation rate. Further, subjecting the *M. smegmatis* DNA repair mutants to Wayne's model of hypoxia showed that loss of Ung and UvrB affected the viability of bacteria. Taken together with the observations made earlier with NER deficient *M. tuberculosis*, these results suggest that NER is

an important DNA repair pathway in mycobacteria to combat commonly encountered DNA damaging agents.

II) Role of adenine DNA glycosylase (MutY) in GO repair pathway.

The oxidative damages to DNA result in the occurrence of 7, 8-dihydro-8-oxoguanine (8-oxoG) in the genome. In eubacteria, repair of such damages is initiated by two major base excision repair enzymes, MutM and MutY. Previous analysis of *fpg* deficient *M. smegmatis* from our lab has shown that the strain is moderately sensitive to hydrogen peroxide and suffers a significant increase in A to G (or T to C) and C to G (or G to C) mutations. It has been reported that in Pseudomonas, another G+C rich organism, loss of *mutY* has a greater impact on spontaneous mutation rate than *fpg*. To further understand the specific role of MutY in GO repair and the DNA repair in mycobacteria in general, we generated a MutY deficient strain of *M. smegmatis*. The MutY deficiency in *M. smegmatis* did not result in either a noteworthy susceptibility to oxidative stress or an increase in the mutation rate. However, the rifampicin resistant isolates of the MutY deficient strain showed distinct mutations in the rifampicin resistance determining region of *rpoB*. Besides the expected C to A (or G to T) mutation, an increase in A to C (or T to G) mutations was also observed. Biochemical characterization of mycobacterial MutY (*M. smegmatis* and *M. tuberculosis*) revealed an expected excision of A opposite 8-oxoG in DNA. Additionally, a detectable excision of G and T opposite 8-oxoG was noted. The MutY formed complexes with DNA containing 8-oxoG×A, 8-oxoG×G or 8-oxoG×T but not 8-oxoG×C pairs. To further understand the interactions of MutY and Fpg in GO repair an antisense technique was employed to down regulate *mutY* in an *fpg* deficient strain of *M. smegmatis*. Expression of antisense significantly reduced the growth rate in bacteria. Thus, in mycobacteria

Fpg and MutY together play an important role in preventing mutations generated due to oxidative stress.

III) Analysis of DNA repair process during hypoxia stress.

Mycobacteria are aerobic bacteria but can adapt to hypoxic conditions that are prevalent within granuloma. Hypoxia is known to cause DNA damages such as single strand breaks, deaminations, etc. It was observed that the loss of DNA repair pathways especially Ung and UvrB resulted in reduced survival of *M. smegmatis* when subjected to *in vitro* hypoxia. Activity analysis for Ung performed from the total protein extract of hypoxically grown bacteria showed reduced activity. Interestingly, when bacteria from hypoxia were recovered aerobically for one hour and then analyzed for Ung activity, the activity was regained. We confirmed the loss of activity in hypoxia was due to reduced protein levels of Ung. Quantitative real-time PCR analysis showed that expression of several DNA repair enzymes was down regulated during hypoxia but the same was restored to mid log levels during recovery stage. To further understand the significance of down regulation of DNA repair enzymes, we cloned *ung* from *M. smegmatis* under a hypoxia specific promoter of *narK2* from *M. tuberculosis* and subjected *M. smegmatis* carrying such a construct to hypoxia. Our results showed that mis-expression of *ung* in hypoxia resulted in reduced bacterial survival. Analysis of Rif^R mutations from mis-expressed strain presented with a significant bias towards A to G mutations. The phenomenon of Ung down regulation was also observed when *M. tuberculosis* H37Ra was subjected to hypoxia, suggesting that down regulation of the DNA repair enzymes plays an important role in the biology of these organisms.