Synopsis of the PhD Thesis Entitled
The Influence of Glycerol and Specific Genes on the Asymmetric Cell Division in Mycobacteria

Submitted by Atul Pradhan (S.R. No. 03-04-00-10-11-14-1-11678)

Background Information: Mycobacteria maintain heterogeneity in cell-length, morphology, and metabolic differences in *in vitro* cultures, infected mice and guinea pigs, and tuberculosis patients. We had reported that ~20-30% of the septating mid-log phase cells of *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, and *Mycobacterium xenopi* undergo highly deviated **a**symmetric **c**onstriction during **d**ivision (**ACD**) to generate short-sized cells (SCs) and normal/long-sized cells (NCs) as sister-daughter cells having differential susceptibility to oxidative/nitrite/antibiotic stress. The remaining 70-80% of the septating cells undergo more-or-less **s**ymmetric **c**onstriction during **d**ivision (**SCD**) generating sister-daughter cells of more-or-less comparable length, as shown by us and other groups. We found that cell-length heterogeneity was achieved in mycobacteria by generating differently sized sister-daughter cells through ACD of NCs and SCD of SCs and NCs, as one of the mechanisms. Subsequently, we discovered Ap_6A as the molecule, which gets secreted into the growth medium, that induces ACD in *M. smegmatis* and *M. tuberculosis* in a paracrine/autocrine manner and the levels of which in change with growth phase. Ap_4A, which was introduced into the medium, was also found to induce ACD.

In *Escherichia coli* and *Bacillus subtilis*, cell-size heterogeneity is affected by the availability of glucose in the culture medium for which UDP-glucose (UDPG) serves as the intracellular proxy for nutrient availability and its concentration decides the cell size during the division. In the present study on *M. smegmatis*, despite the absence of the orthologues of UgtP of *B. subtilis* and OpgH of *E. coli* that are involved in UDPG metabolism, we examined the influence of glycerol on the proportions of cells undergoing division by ACD and SCD. Further, we identified the genes that influence the synthesis and degradation of Ap_4A, which is another Ap_nA, probably the precursor to Ap_6A, also found to be involved in inducing ACD.

Layout of the Thesis: The **Chapter 1**, which forms the **Introduction** to the thesis, gives an extensive literature survey on different areas of research in the bacterial physiology that are linked to the present study. These areas of research include bacterial cell division, different mode of cell division in mycobacteria, cell division related proteins in bacteria, relation between cell division and nutrient status, different carbon sources used by bacteria for survival, estimation of free

glycerol in the medium, genes and its biochemical activity, related to diadenosine polyphosphates synthesis and phosphorylation in bacteria.

The **Chapter 2** forms the **Material and Methods** used in the present study. Here a detailed description of the methods used to study growth phase of bacteria, cell division and bioassay used to score for the proportion of cells undergoing symmetric and asymmetric constriction during division, assay for free glycerol concentration in the medium, Ap_6A exposure to *M. smegmatis* (*Msm*) and its effect on ACD/SCD, RNA extraction and qPCR analysis, Protein overexpression of MSMEG_2932 and its biochemical activity using different substrate followed by identification and quantitation of different products with the help of HPLC.

The **Chapter 3** forms the first data chapter that presents the results on the effects of growth phase of *Msm* and free glycerol concentration in the medium on the proportions of cells dividing by SCD and ACD. Here we have shown as to how the changes in the glycerol (main carbon source) concentration with the growth phase affect the proportion of cells dividing by SCD and ACD in the population. The population shows change in the proportion of SCD and ACD. The proportion could be maintained by maintaining free glycerol concentration in the medium. We had also tested the effect of spent medium from different growth phases on the ACD:SCD proportions.

The **Chapter 4** forms the data chapter that presents the levels of expression of MSMEG_2932, MSMEG_2933, and MSMEG_2936, which were found to influence ACD. Hence the proportions of cells dividing by ACD:SCD, in a growth phase dependent manner, were determined using the respective gene knockout mutants. The ACD:SCD proportion change with growth phase and glycerol concentration in the medium was more or less reversed or unaffected in the KO strains. Quantitative PCR was performed for the expression levels of the three genes at 0.8 OD and in the Ap_6A-exposed cells.

This data chapter also presents the results of MSMEG_2932 protein overexpression, purification and study of activity as an Ap_4A hydrolase/phosphorylase and as a Ap_4A synthase at a variety of conditions involving different pH, buffers, temperatures, and divalent ions. Thus, the present study for the first time has shown the influence of glycerol on ACD:SCD proportions in the dividing population of *Msm* cells. The present study also reports the *in vitro* synthesis of Ap_4A by MSMEG_2932, using ATP as the substrate.

The thesis is concluded with a section on general discussion, salient findings from the work, followed by an extensive bibliography.