

## Preface

The work of this thesis has its origin in the reiterative isolation of DNA segment conferring low level multiple resistance/tolerance to unrelated antibiotics, invariably containing *relA* and *rumA* genes, from at least three independent *Escherichia coli* libraries constructed in the lab. The impetus led us to investigate the mechanism of tolerance. As the host *Escherichia coli* DH5 $\alpha$  is wild type with respect to genes carried on the plasmid DNA, it was anticipated that the mechanism of tolerance is most probably multiple copy function of the gene(s). Multicopy expression of gene(s) brings to the fore the latent moonlighting activities not obvious otherwise in single copy state (Patrick *et al.*, 2007, Li *et al.*, 2004). For this reason and the reason that a strong genetic component is involved in intrinsic tolerance to antibiotics, we decided to investigate the cause of the phenotype of tolerance. The results obtained in the work indicate that multiple copies of *relA* expressed from its own promoter and not from the heterologous promoter is enough to confer multiple antibiotic tolerance, however only in DH5 $\alpha$ . Since this phenotype is strain specific (not observed in strains JM101, and MG1655), we did not find out the cause of the strain specificity.

Nevertheless, the second aspect of the two genes interested us even more. RumA possesses the catalytic activity of transferring, in SAM-dependent reaction, methyl moiety to U1939 of 23S rRNA converting it into one of the two Ts' present in ribosomal RNA; the other being U747 in 23S rRNA, which is methylated by RumB. U1939 is present in the structurally conserved, functionally important loop of 23S rRNA. Its functional importance lies in the fact that in the 3D structure of ribosome in complex with tRNA, the U1939 loop protrudes into the major groove of tRNA in the A-site at the end of the acceptor arm; a vantage point for sensing the binding of unacylated tRNA at the A-site during nutrition starvation/stringent response (Agarwalla *et al.*, 2002). RelA is responsible for synthesis of two compounds ppGpp, and pppGpp, collectively called (p)ppGpp, in response to nutrient starvation by the mechanism that involves binding of uncharged tRNA to the A-site on the ribosome in complex with mRNA. The ribosome bound RelA protein is activated catalytically in the presence of ribosomal protein L11, causing synthesis of (p)ppGpp. The functional intimacy is also reflected in their being together in the

genome of several  $\gamma$ -proteobacteria. Persaud *et al.*, (2010) deleted the complete ORF of *rumA* (Persaud *et al.*, 2010). Since the mutant was not majorly affected for growth, the authors proclaimed the gene inessential. However the same deletion also removed all the promoters of *relA* which are in *rumA* (Nakagawa *et al.*, 2006). Thus, if any aspect of regulation of *relA* is affected it would not be manifest. In order to address the role of *rumA*, we decided to carry out SDM of catalytic site of RumA and also generate insertion mutation in *rumA* upstream of promoter sequences of *relA*. The third objective (Chapter-6) demonstrates the importance of upstream *rumA* DNA but not *rumA per se* in regulation of expression of *relA*.

## References

- Agarwalla, S., J.T. Kealey, D.V. Santi & R.M. Stroud, (2002) Characterization of the 23 S ribosomal RNA m<sup>5</sup>U1939 methyltransferase from Escherichia coli. *Journal of Biological Chemistry* **277**: 8835-8840.
- Li, X., M. Zolli-Juran, J.D. Cechetto, D.M. Daigle, G.D. Wright & E.D. Brown, (2004) Multicopy suppressors for novel antibacterial compounds reveal targets and drug efflux susceptibility. *Chemistry & biology* **11**: 1423-1430.
- Nakagawa, A., T. Oshima & H. Mori, (2006) Identification and characterization of a second, inducible promoter of *relA* in Escherichia coli. *Genes & genetic systems* **81**: 299-310.
- Patrick, W.M., E.M. Quandt, D.B. Swartzlander & I. Matsumura, (2007) Multicopy suppression underpins metabolic evolvability. *Molecular biology and evolution* **24**: 2716-2722.
- Persaud, C., Y. Lu, A. Vila-Sanjurjo, J.L. Campbell, J. Finley & M. O'Connor, (2010) Mutagenesis of the modified bases, m<sup>5</sup>U1939 and  $\psi$ 2504, in *Escherichia coli* 23S rRNA. *Biochemical and biophysical research communications* **392**: 223-227.