

## **CONTROL OF THE SYNTHESIS OF RIBOSOMES IN *MYCOBACTERIUM FORTUITUM***

M.C. Nuñez<sup>1</sup>, M.C. Menendez<sup>1</sup>, R.A. Cox<sup>2</sup> & M.J. Garcia<sup>1</sup>

1) Facultad de Medicina. Universidad Autonoma de Madrid. Madrid. SPAIN

2) National Institute for Medical Research. Medical Research Council. London. UNITED KINGDOM

The *purpose of this study* was to test the value of quantification using real-time PCR in the analysis of the kinetics and control of the synthesis of ribosomes, in rapidly growing mycobacteria carrying two (*rrnA* and *rrnB*) ribosomal RNA operons per genome. The analysis was undertaken to provide insights into the control of the bacterial growth rate.

*Methods.* *M. fortuitum* were recovered from cultures in liquid medium at five different points along the growth curve (from exponential to stationary phases). The mycobacterial total RNA was isolated, purified, and retro-transcribed using random-hexamers to obtain cDNA. Quantitative real-time PCR was applied using the Light-Cycler System (Roche) and SYBR green DNA intercalator. The species quantified included mature 16S rRNA, pre-*rrn* products (*rrnA*-P1; *rrnA*-PCL1; *rrnB*-P1), and mRNA coding for two ribosomal proteins S12 [*rpsL*] and L7 [*rplL*] corresponding to the small and large subunits of the ribosome respectively.

*Results.* Relative to 16S rRNA the amounts of the pre-*rrn* products were similar during exponential phase and decreased during stationary phase. The amounts of mRNA encoding S12 and L7 also diminished on entry into stationary phase.

*Conclusion:* The contributions of *rrnA* and *rrnB* operons to 16SrRNA synthesis were found to be different. The *rrnA* operon was found to make the major contribution to 16S rRNA synthesis during both exponential and stationary phases of growth; the PCL1 promoter was found to be more active than the P1 promoter. In contrast, the contribution of the *rrnB* promoter was less during stationary phase than in exponential phase.