ABSTRACT

The Chaperonins (HSP60) constitute a family of essential molecular chaperone proteins that function as key players in the cellular proteostasis network. They are responsible for the folding and function of ~10 – 15% of the cellular proteins under normal conditions, which increases to ~ 30% under conditions of stress, such as heat shock. Group I chaperonins, that are predominantly found in the bacterial cytosol, mitochondrial lumen and in chloroplasts, function in association with the HSP10 (HSP20 in chloroplasts) family of proteins, also known as the co-chaperonins. The bacterial homologs of HSP60 and HSP10 are denoted by Cpn60 and Cpn10 respectively, to distinguish them from the organellar homologs. Most of the studies investigating the structure and function of bacterial chaperonin systems have been centred around the *E. coli* chaperonin system consisting of GroES (Cpn10) and GroEL (Cpn60). Until recently, the GroES-GroEL system was deemed as the prototypical bacterial chaperonin systems vis-à-vis structure, function and mechanisms of action. However, recent investigation of diverse chaperonin systems have illustrated that the biochemical and biophysical properties of chaperonins may not be universal. Mycobacteria, a genus containing some notorious pathogens, curiously possess two chaperonin genes, *cpn60.1* and *cpn60.2*, but only one co-chaperonin gene, *cpn10*. The protein products of the Mycobacterial *cpn* genes are particularly enigmatic in that they possess multiple moonlighting functions, while their molecular chaperone functions have not been illustrated with certainty. In this thesis, we sought to explore the molecular chaperone function of the chaperonin systems, Cpn10-Cpn60.1 and Cpn10-Cpn60.2, from two pathogenic *Mycobacterium* species, *M. tuberculosis* and *M. marinum*.

We began by analysing the similarities and differences between the *E. coli* and Mycobacterial chaperonin and co-chaperonin proteins at the sequence and structural levels. We specifically probed the presence of conserved motifs and domains critical for chaperonin function in the Mycobacterial homologs. Thereafter, we evaluated the ability of the Mycobacterial chaperonin systems to reinstate intracellular chaperonin function in an *E. coli* experimental strain depleted

of endogenous GroES and GroEL. We initially investigated the chaperonin function by evaluating the ability of the Mycobacterial chaperonin systems to restore cell viability and proliferation in the *E. coli* model. We found that the Cpn10-Cpn60.1 systems could only support cell survival, while the Cpn10-Cpn60.2 systems could also augment cell proliferation. Our findings suggest that, in Mycobacteria, both the chaperonin systems possess intracellular chaperonin function but with subtle differences amongst the two systems. We subsequently proceeded with chaperonin-assisted protein-folding assays to probe the ability of the chaperonin systems in preventing the aggregation and augmenting the folding of MetK, an obligate GroEL client and an essential *E. coli* protein. The *in vivo* functional assays illustrated that the Cpn10-Cpn60.1 systems are adept at preventing MetK aggregation, while Cpn10-Cpn60.2 are proficient at supporting both aggregation prevention and productive folding. Furthermore, in line with a previous report on phosphorylation-induced oligomerisation of Cpn60.1, we decided to evaluate the impact of Cpn60.1 phosphorylation on its chaperonin function. Our findings suggest that phosphorylation at Ser75 and Ser393 augments the chaperonin function of the *M. tuberculosis* Cpn60.1, allowing it to support cell proliferation in the *E. coli* model. This appears to be an adaptive mechanism to regulate the overall intracellular chaperonin capacity under normal conditions, which may be enhanced by Cpn60.1 phosphorylation to improve cellular fitness under conditions of stress.

In conclusion, our findings illustrate the intracellular molecular chaperone function of the Mycobacterial chaperonin systems. We hereby establish that both the chaperonin systems of *M. tuberculosis* and *M. marinum* (and by extrapolation, possibly of all Mycobacteria) are adept at canonical chaperonin function. On the basis of our findings, we propose that Cpn10-Cpn60.2 represents the housekeeping chaperonin system in Mycobacteria, with Cpn10-Cpn60.1 supplementing the intracellular chaperonin capacity as per the cellular requirements.