

TABLE OF CONTENTS

1. INTRODUCTION	3
1.1. Human tuberculosis	3
1.2. <i>M. bovis</i> bacillus Calmette-Guérin (BCG)	5
1.3. The mycobacterial cell wall	6
1.3.1. The peptidoglycan networks.....	7
1.3.2. The arabinogalactan layer.....	8
1.3.3. Mycolic acids biosynthesis and transport	9
1.3.4. Cell wall mycoloylation	12
1.3.5. Phosphatidylinositol mannoside (PIM), lipomannan (LM) and lipoarabinomannan (LAM).....	12
1.3.6. Phthiocerol/phthiodiolone dimycocerosate (PDIM) and phenolic glycolipid (PGL) 14	
1.4. Propionyl-CoA-assimilating pathways	21
1.4.1. Generation of propionyl-CoA	21
1.4.2. Toxic effects of propionyl-CoA.....	23
1.4.3. Propionyl-CoA detoxification pathways	24
1.5. TB chemotherapy	26
1.5.1. Drug-susceptible TB.....	26
1.5.1.1. Mode of action of INH and ethionamide (ETH).....	26
1.5.1.2. Mode of action of RIF, PZA and EMB	27
1.5.2. Drug-resistant TB.....	29
1.5.3. Recently developed/repurposed drugs	30
1.5.3.1. Q203 and terminating oxidases of mycobacterial ETC.....	31
1.5.3.2. Bedaquiline and F ₀ F ₁ ATP synthase.....	33
1.5.3.3. Clofazimine and mycobacterial NADH dehydrogenases (NDHs).....	34
1.5.3.4. NDHs inhibitors.....	36
1.5.3.5. Oxazolidinones and nitroimidazoles	36
1.6. Mycobacterial nonreplicating “dormancy”	37
1.6.1. DosR-dependent dormancy establishment.....	37
1.6.2. Requirement of DosR for mycobacterial survival under hypoxia.....	39
1.6.3. Dormancy regulon induction under NO, CO and drug exposure.....	39
1.6.4. Sensor kinases DosT and DosS.....	40
1.6.5. Induction of DosR regulon under normal condition	42
1.6.6. Dormancy and phenotypic drug resistance	42
1.6.7. The mycobacterial respiratory chain under hypoxia.....	43
1.6.7.1. Initiation of ETC	43
1.6.7.2. Terminal oxidases and electron acceptors	44
1.6.7.3. Eradication of nonreplicating mycobacteria by targeting ETC	44
1.7. Mycobacterial survival strategies under stresses	45
1.7.1. Mycobacterial adaptation into starvation	45
1.7.2. Stringent response	46
1.7.3. Biofilm formation	46

1.8. Mycobacterial chaperonin 60.1 (Cpn60.1/GroEL1)	48
1.8.1. Mycobacterial chaperonins.....	48
1.8.2. Expression and function of Cpn60.1.....	49
1.9. Nucleoid-associated proteins (NAPs)	50
2. OBJECTIVES	53
2.1. Role of Cpn60.1 in mycobacterial dormancy establishment and antibiotic susceptibility	53
2.2. Role of Cpn60.1 in <i>M. bovis</i> BCG biofilm formation	53
3. MATERIALS AND METHODS	54
3.1. <i>M. bovis</i> BCG strains	54
3.2. Media	54
3.2.1. 7H9 medium with 0.05% Tween 80	54
3.2.2. 7H9 medium with 0.2% (vol/vol) glycerol.....	55
3.2.3. DTA medium.....	55
3.2.4. Sauton's medium	56
3.2.5. 7H11 agar	56
3.3. Wayne dormancy model	57
3.4. Biofilm growth	57
3.5. Growth quantification	58
3.5.1. Growth determination in the Wayne dormancy model	58
3.5.2. Growth of <i>M. bovis</i> BCG strains under Sauton's medium	58
3.5.3. Growth in DTA medium.....	58
3.6. RNA extraction, DNase treatment, reverse transcription (RT) and real-time polymerase chain reaction (PCR)	59
3.6.1. RNA extraction	59
3.6.2. DNase treatment and RNA clean-up	59
3.6.3. Reverse transcription (RT)	60
3.6.4. Real-time PCR	61
3.7. Antibiotic susceptibility assays: bactericidal or bacteriostatic assessment	63
3.7.1. Antibiotic susceptibility assay in liquid cultures	63
3.7.2. Biofilm drug susceptibility assay.....	63
3.7.3. Minimal inhibitory concentration (MIC) determination	64
3.8. Adenosine triphosphate (ATP) determination	64
3.9. Determination of reactive oxygen species (ROS)	64
3.10. Construction of <i>M. bovis</i> BCG harboring <i>pfadD26-luxAB</i>	65
3.10.1. Preparation of vector.....	65
3.10.2. Preparation of insert.....	67

3.10.3. In-fusion cloning and transformation	72
3.10.4. Miniprep, PCR screening and sequencing	73
3.10.5. Transformation and selection of recombinant <i>M. bovis</i> BCG strains.....	77
3.11. Bioluminescence detection	77
3.12. Determination of methylglyoxal-protein adduct by enzyme-linked immunosorbent assay (ELISA).....	78
3.13. Bradford assay	78
3.14. Quantification of carbonyl group-bearing metabolites and pyruvate	79
3.15. Succinate quantification	80
3.16. Acetate determination	80
3.17. Glutamate/glutamine measurement	81
3.18. MIC of methylglyoxal (MG).....	81
3.19. MG on <i>M. bovis</i> BCG viability	81
3.20. Oxygen consumption assay	81
3.21. Membrane potential measurement.....	82
3.22. Drug treatment in a settling culture model	82
3.23. Lipid analysis.....	83
3.24. Proteomic analysis	83
3.25. Statistical analysis and graph preparation	83
4. RESULTS.....	85
4.1. Role of Cpn60.1 in mycobacterial survival and antibiotic susceptibility under hypoxia.....	85
4.1.1. Cpn60.1, PDIM and PGL are nonessential for mycobacterial survival under hypoxia	85
4.1.2. Loss of Cpn60.1 triggers an aerobic induction of genes in the DosR regulon but compromised their induction under hypoxia.....	86
4.1.3. Loss of Cpn60.1 potentiates INH's bactericidal activity under hypoxia, independently of PDIM/PGL alteration	88
4.2. INH's bactericidal activity involves electron transport chain (ETC) perturbation.....	91
4.2.1. INH as well as ETH increases mycobacterial ATP levels quickly, independently of Cpn60.1	91
4.2.2. The ATP increase is not linked to a general cell envelope stress response	94
4.2.3. The ATP increase is proportional to INH concentration and correlated to the drug's bactericidal activity	95

4.2.4.	The INH-induced ATP increase is concomitant with enhanced oxygen consumption.....	97
4.2.5.	Q203 and bedaquiline compromise the INH-induced ATP increase and bactericidal activity	100
4.2.6.	Proteomic analysis	102
4.2.7.	ROS determination and effect of antioxidant NAC and TEMPOL	103
4.2.8.	NAC rescues the <i>Δcpn60.1</i> strain from INH's killing under hypoxia.....	106
4.2.9.	Chemical inhibition of NDHs and SDHs protects mycobacteria from INH's killing	107
4.2.10.	INH dissipates mycobacterial membrane potential	110
4.2.11.	Inhibition of cytochrome <i>bd</i> oxidase reduces cell recovery under INH challenge	112
4.3.	Cpn60.1 (GroEL1) contributes to mycobacterial Crabtree effect: implications for biofilm formation.....	114
4.3.1.	Defective PDIM and PGL production partially contributes to the biofilm defect of the <i>Δcpn60.1 M. bovis</i> BCG	114
4.3.2.	PDIM and PGL contribute to reduced drug susceptibility also in biofilm .	116
4.3.3.	The growth of the <i>Δcpn60.1</i> strain under excess glycolytic carbon source is largely compromised	116
4.3.4.	Improved biofilm growth of the <i>Δcpn60.1</i> strain by reducing glycerol concentration	119
4.3.5.	Accumulation of methylglyoxal accounts for the <i>Δcpn60.1</i> growth defect	121
4.3.6.	Problematic Crabtree effect in the <i>Δcpn60.1</i> strain under excess glycerol	123
4.3.7.	Proteomic analysis	127
4.3.8.	The <i>Δcpn60.1</i> strain secretes more glutamate	129
4.4.	Cpn60.1 is required for respiratory adaptation in response to vancomycin and Q203.....	131
4.4.1.	Defective vancomycin-induced ATP downregulation in the absence of Cpn60.1	131
4.4.2.	Compromised respiratory adaptation to cytochrome <i>bc₁</i> inhibition in the absence of Cpn60.1	131
4.5.	Glycerol modulates mycobacterial biofilm and lipid composition.....	133
4.5.1.	Glycerol modulates mycobacterial biofilm	133
4.5.2.	Glycerol regulates PDIM production.....	134
4.5.3.	Glycerol affects the production of PGL, GroMM, TMM, TDM and PIM ...	136
5.	DISCUSSION	138
5.1.	Regulation of DosR regulon by Cpn60.1: a protein chaperonin and/or nucleoid-associated protein (NAP)?	138
5.2.	Isoniazid bactericidal mechanism: a role for Cpn60.1 in the protection against isoniazid under hypoxia	139
5.3.	Mycobacterial biofilm growth, the Crabtree effect and Cpn60.1	144

5.4. Cpn60.1 participates in mycobacterial respiratory adaptation under stresses	151
6. CONCLUSIONS AND PERSPECTIVES.....	153
7. REFERENCE.....	155
8. ANNEX	180
8.1. Annex 1. Proteomic procedure	180
8.1.1. Protein extraction	180
8.1.2. Separation of peptides	180
8.1.3. SWATH acquisition	180
8.2. Annex 2. Selected proteins with altered expression relative to no drug control after 7 hours treatment with 0.4 µg/ml INH	182
8.3. Annex 3. Selected proteins with altered expression relative to INH after 7 hours treatment with Q203 plus INH.....	184
8.4. Annex 4. WT proteomic comparison between 6% glycerol and 0.2% glycerol Sauton's medium.....	187
8.5. Annex 5. Proteomic comparison between the WT and Δcpn60.1 BCG under 4% glycerol Sauton's medium	197