



Toxicogenomic response of *Mycobacterium bovis* to hydrogen peroxide



Juyoung Jung¹, Chantal Nde², Freshteh Toghrol³, William E. Bentley², and Hyeung-Jin Jang^{1,2}

¹ Department of Biochemistry, College of Oriental Medicine, Kyung Hee University, Seoul 130–701, Republic of Korea, ² Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, Maryland 20742, USA, ³ Microarray Research Laboratory, Biological and Economic Analysis Division, Office of Pesticide Programs, U. S. Environmental Protection Agency, Fort Meade, Maryland 20755, USA

ABSTRACT

Background: *Mycobacterium bovis* BCG strain Pasteur 1173P2 responds with adaptive and protective strategies against oxidative stress. Despite advances in our understanding of the responses to oxidative stress in many specific cases, the connectivity between targeted protective genes and the rest of cell metabolism remains obscure.

Results: The *furA* gene in *Mycobacterium bovis*, a pleiotropic regulator that couples iron metabolism to the oxidative stress response was involved in the response to hydrogen peroxide stress. There were also increased levels of catalase/ peroxidase (KatG) and the biosynthesis operon of mycobactin. This study revealed significant upregulation of the oxidative response group of *M. bovis*, amino acid transport and metabolism, defense mechanisms, DNA replication, recombination and repair, and downregulation of cell cycle control, mitosis, and meiosis, lipid transport and metabolism, and cell wall/membrane biogenesis.

Conclusions: This study shows that the treatment of *M. bovis* BCG with hydrogen peroxide induces iron acquisition related genes and oxidative stress response genes within one hour of treatment.

Mycobacterium bovis BCG genes that showed statistically significant mRNA level changes upon either 10 or 60 min exposure to hydrogen peroxide

Transcript level comparison of *Mycobacterium bovis* BCG genes between real-time PCR and microarray analyses

| Gene Annotation | 10min | | 60min | | Description | Functional group |
|---|---------|------|---------|------|--|--|
| | P-value | Fold | P-value | Fold | | |
| Group I: Upregulation (10min) - Upregulation (60 min) 7 genes | | | | | | |
| BCC_1947G | 0.00124 | 5.5 | 0.00124 | 2.7 | Catalase-peroxidase, peroxidase 7 katG | Inorganic ion transport and metabolism |
| BCC_1948G | 0.00111 | 4.9 | 0.00111 | 2.3 | Ferric uptake regulation protein furA | Inorganic ion transport and metabolism |
| BCC_2396G | 0.00381 | 4.5 | 0.00381 | 1.9 | Polyketide synthase mbtC | Secondary metabolites biosynthesis, transport and catabolism |
| BCC_2397C | 0.00139 | 3.6 | 0.00139 | 2.0 | l-phenylalanine synthase mbtB | Secondary metabolites biosynthesis, transport and catabolism |
| BCC_3009G | 0.00589 | 3.4 | 0.00589 | 1.5 | putative 3-isopropylmalate dehydratase large subunit leuC | Amino acid transport and metabolism |
| BCC_1712G | 0.0124 | 1.9 | 0.0124 | 1.6 | putative transcriptional regulatory protein | Inorganic ion transport and metabolism |
| BCC_0020 | 0.028 | 1.6 | 0.028 | 1.5 | Thioredoxin hxc (TRX) (MPT4) | Posttranslational modification, protein turnover, chaperones |
| Group II: Upregulation (10min) - No change (60 min) 11 genes | | | | | | |
| BCC_2395G | 0.00811 | 3.2 | | | Polyketide synthase mbtD | Secondary metabolites biosynthesis, transport and catabolism |
| BCC_3008G | 0.0278 | 2.4 | | | putative 3-isopropylmalate dehydratase small subunit leuD | Amino acid transport and metabolism |
| BCC_2405G | 0.00508 | 2.1 | | | putative isochlorogenic acid synthase mbtI | Amino acid transport and metabolism |
| BCC_2394G | 0.0132 | 2.0 | | | Peptide synthetase mbtE | Secondary metabolites biosynthesis, transport and catabolism |
| BCC_1590G | 0.0235 | 1.9 | | | putative polyketide synthase associated protein mbtA | Secondary metabolites biosynthesis, transport and catabolism |
| BCC_2140 | 0.0156 | 1.9 | | | PPE family protein | |
| BCC_0328 | 0.0246 | 1.7 | | | PPE family protein | |
| BCC_1411 | 0.0273 | 1.7 | | | inhibitor drug-transport transmembrane ATP-binding protein ABC transporter | Defense mechanism |
| BCC_1410 | 0.00425 | 1.7 | | | putative drug-transport transmembrane ATP-binding protein ABC transporter | Defense mechanism |
| BCC_0862G | 0.00822 | 1.5 | | | putative 3-hydroxyacyl-CoA dehydrogenase rnaB | Lipid transport and metabolism |
| BCC_1408 | 0.0207 | 1.5 | | | putative acyl carrier protein | Lipid transport and metabolism |
| Group III: No change (10 min) - Upregulation (60min) 3 genes | | | | | | |
| BCC_3227G | | | 0.0205 | 1.8 | putative ATP-dependent DNA helicase | DNA replication, recombination and repair |
| BCC_1377G | | | 0.00117 | 1.6 | putative methylated DNA-protein-cysteine Methyltransferase ogf | DNA replication, recombination and repair |
| BCC_3228G | | | 0.00607 | 1.6 | putative ATP-dependent DNA helicase | DNA replication, recombination and repair |
| Group IV: No change (10 min) - Downregulation (60min) 14 genes | | | | | | |
| BCC_0029G | | | 0.011 | -1.5 | putative chromosome partitioning protein parB | Transcription |
| BCC_0024G | | | 0.0196 | -1.8 | putative chromosome partitioning protein parA | Cell cycle control, mitosis and meiosis |
| BCC_0025G | | | 0.015 | -1.5 | putative glucose-inhibited division protein B GID | Cell wall/membrane biogenesis |
| BCC_0119 | | | 0.0254 | -1.5 | putative hydrogenase hsdG | Energy production and conversion |
| BCC_0567 | | | 0.0169 | -1.7 | putative transmembrane protein | |
| BCC_1457G | | | 0.0222 | -1.6 | PE-PGRS family protein | |
| BCC_1747 | | | 0.0334 | -1.6 | putative inhibition inhibitor protein | Cell cycle control, mitosis and meiosis |
| BCC_1751 | | | 0.00439 | -1.6 | putative cytidylate kinase omk | Nucleotide transport and metabolism |
| BCC_1754 | | | 0.021 | -1.6 | putative 3-hydroxybutyryl-CoA dehydrogenase hsdD2 | Lipid transport and metabolism |
| BCC_2969G | | | 0.0419 | -1.5 | putative fatty acid-CoA ligase hsdD2 | Lipid transport and metabolism |
| BCC_3075G | | | 0.026 | -1.6 | putative chromosome partitioning protein parB | Transcription |
| BCC_3076G | | | 0.0283 | -1.7 | putative chromosome partitioning protein parA | Cell cycle control, mitosis and meiosis |
| BCC_3077G | | | 0.0134 | -1.5 | putative glucose-inhibited division protein B gid | Cell wall/membrane biogenesis |
| BCC_3078G | | | 0.0072 | -1.5 | hypothetical protein similar to pig protein | General function prediction only |

| Gene | mRNA level change with microarray | | mRNA level change with real-time PCR | | Sense primer sequence | Antisense primer sequence |
|-----------|-----------------------------------|-------|--------------------------------------|-------------|---|---|
| | Fold change | | Fold change | | | |
| | 10min | 60min | 10min | 60min | | |
| BCC_1947G | 5.5 | 2.7 | 18.0 (± 0.1) | 5.5 (± 0.3) | 5'- AAC ATC AAA GTG TCC TTC GCC GAC -3' | 5'- GCA AAG GAT TCC ACC TCG GTT TGT -3' |
| BCC_1948G | 4.9 | 2.3 | 10.3 (± 0.1) | 2.9 (± 0.2) | 5'- TCG GAC CAT AAC GGG TCG CTO TT -3' | 5'- GAT GTG ATC GCG AAG TGT CGG ATA -3' |
| BCC_2396G | 4.5 | 1.9 | 7.1 (± 0.1) | 5.3 (± 0.2) | 5'- CTT TCA CAC GCG GGT TCA AGG TAT -3' | 5'- TCG TCG TTG GAG AAC TCG ACC AAA -3' |
| BCC_2397C | 3.6 | 2.0 | 7.5 (± 0.1) | 4.8 (± 0.3) | 5'- ATC TCG CGA CTT TCC CAT CAG TT -3' | 5'- GTC AAC GGA AGT TCG AAT ACC GCA -3' |
| BCC_3009G | 3.4 | 1.5 | 7.1 (± 0.1) | 2.5 (± 0.4) | 5'- AAA GCA TTG GCG TAC ATG GCC TT -3' | 5'- TTC AAT GCG ACC GTT GGT ACA GCA -3' |
| BCC_0920 | 1.6 | 1.5 | 4.5 (± 0.1) | 3.3 (± 0.1) | 5'- AAA GTT ACC GAC GCA TCC TTG GCG -3' | 5'- TAG CAT CTT GCA AGG TCG AGA GCA -3' |
| BCC_2395G | 3.2 | - | 11.7 (± 0.1) | - | 5'- AGG ACT ACC ACC TGG TAG GAA ACA -3' | 5'- TCG GCA TTA CCG GAG TAA AGA ACT -3' |
| BCC_3008G | 2.4 | - | 3.0 (± 0.6) | - | 5'- AAG CCT TTC ACA CCC ACT CTC GTA -3' | 5'- TCA GAA AGA CCG CGG GAA TGA TCT -3' |
| BCC_2400G | 2.1 | - | 5.9 (± 0.4) | - | 5'- TGG CTC GTG ATG ACC TGG AAT GAA -3' | 5'- TAA TCT CCT CAA GCG AAG ACC GCA -3' |
| BCC_2394G | 2.0 | - | 2.6 (± 0.2) | - | 5'- FAT TGG AGG GAC GCA TGT CGC ATA -3' | 5'- TTC GGA ATC CCG GAA TCT TGA CCT -3' |
| BCC_1410 | 1.7 | - | 5.0 (± 0.2) | - | 5'- GGA TCC GGT GAA TTT GTT GCC GTT -3' | 5'- AAT TCG GTT TCG TCG AGT GTG ACT -3' |
| 16S rRNA* | 1.0 | 1.0 | 1.0 | 1.0 | 5'- TGC AAG TCG AAC GGA AAG GTC TCT -3' | 5'- AAG ACA TGC ATC CCG TCG TCC TAT -3' |

* The real time PCR results are the mean of three biological replicates with three technical replicates for each gene.

MATERIAL AND METHOD

• *M. bovis* exposed to hydrogen peroxide (0.5mM) for 10 and 60min

• Affymetrix *M. bovis* BCG custom genechip analysis

• Statistical analysis of microarray data

- p-value for the t-test ≤ 0.05

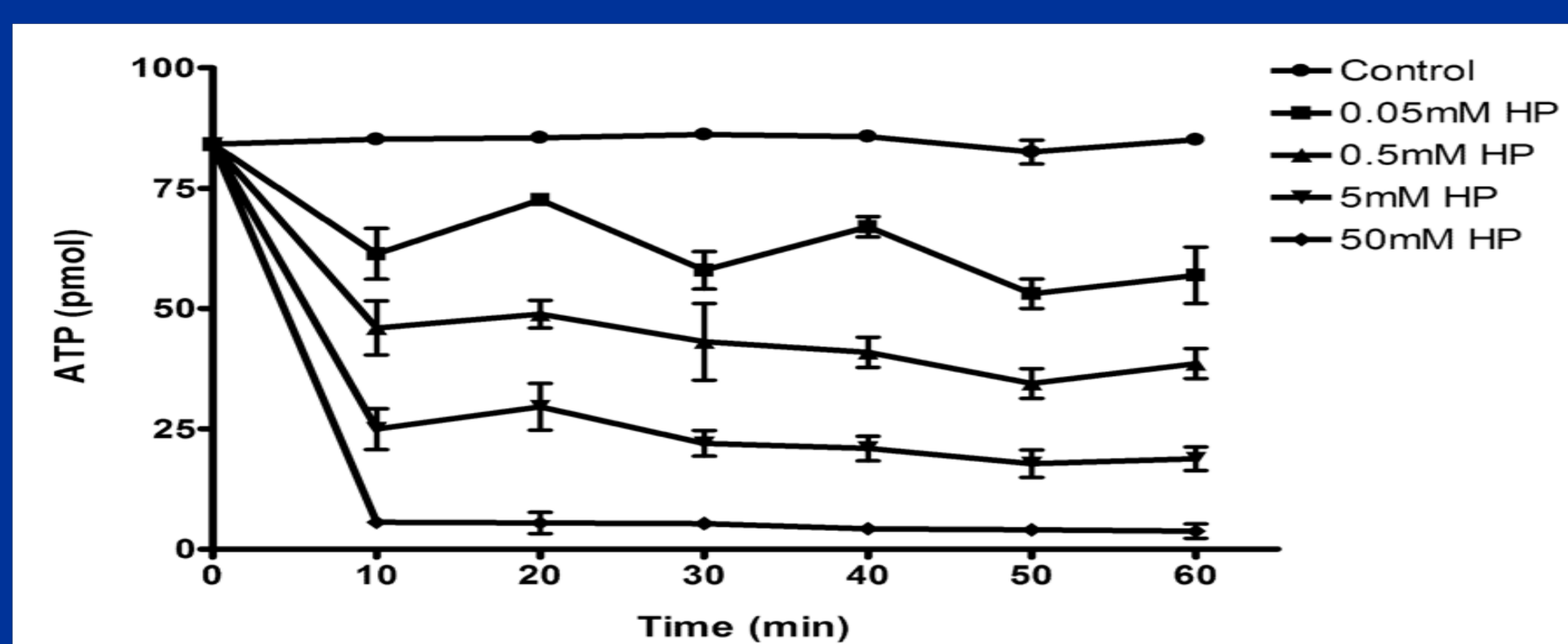
- Fold change in transcript level ≥ 2.0

- Presence or marginal calls ≥ 50% replicates on both the experimental and control sets

• Quantitative real-time PCR used for the validation of the microarray data

RESULTS AND DISCUSSION

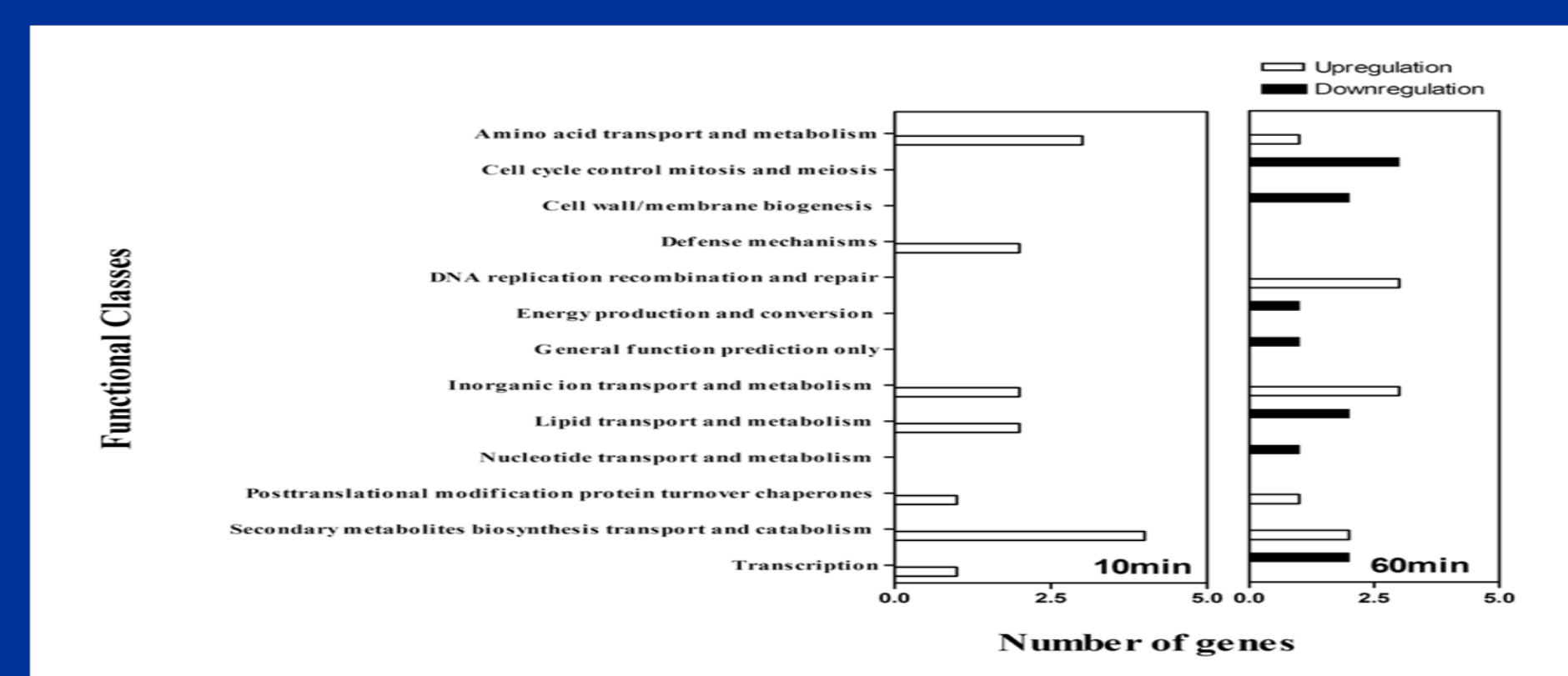
Growth Inhibition of *M. bovis* BCG treated with hydrogen peroxide



* Changes in the amount of ATP produced by the growth culture of *M. bovis* BCG after treatment with hydrogen peroxide were measured in 10 minute intervals for one hour. 0 mM, control (filled circle), 0.05 mM (filled square), 0.5 mM (filled triangle), 5 mM (inverted filled triangles) and 50 mM (filled diamond).

* The genes were grouped based on their regulation directions upon 10 and 60 min exposures.

Functional classification of genes showing statistically significant upregulation and downregulation in transcription levels after 10 and 60 minutes exposure to 0.5mM hydrogen peroxide



* Upregulation (mRNA level changes of 1.5 fold or more, empty square) and downregulation (filled square).