Prof. Dr. Charles J. Thompson
Biozentrum, University of Basel
Division of Molecular Microbiology
Klingelbergstrasse 70
4056 Basel
charles.thompson@ubc.ca

4049-069384
01.10.2002-31.03.2006

Prof. Dr. Stephan Grzesiek
Biozentrum, Division of Stuctural Biology, University of Basel
Stephan.grzesiek@unibas.ch

Overcoming intrinsic multidrug resistance to discover and expand the repertoire of antibiotics active against mycobacterial pathogens

Objectives Mycobacterial infections are one of the leading causes of human mortality. Chemotherapeutic options to treat tuberculosis are severely restricted by the intrinsic resistance of these bacteria to the majority of clinically applied antibiotics. This resistance is partially provided by the low permeability of their unique cell envelope. Work is underway to discover and design novel antimycobacterial drugs.

Conclusions Ligation of mycolic acids to structural components of the mycobacterial cell wall generates a hydrophobic, impermeable barrier that provides resistance to toxic compounds such as antibiotics. The studies of *Mycobacterium smegmatis* showed that FbpA-dependent biosynthesis of trehalose dimycolate is required for the intrinsic multidrug resistance, cell wall structure and colonial morphology of *Mycobacterium smegmatis*. These findings support the concept that FbpA-specific inhibitors, alone or in combination with other antibiotics, could provide an effective treatment for tuberculosis and other mycobacterial diseases.
The *whiB7* gene of *M. tuberculosis* was found to be a central regulator that coordinates the expression of a family of resistance genes able to inactivate antibiotics that have penetrated into the cytoplasm. Therefore, since a single regulatory protein (*WhiB7*) activates resistance to multiple drugs in mycobacterial pathogens, components of the *whiB7* system could serve as novel drug targets (i.e. inhibitors), rendering *M. tuberculosis* or multidrug-resistant derivatives more antibiotic-sensitive.

Main results and findings

Part I Ligation of mycolic acids to structural components of the mycobacterial cell wall generates a hydrophobic, impermeable barrier that provides resistance to toxic compounds such as antibiotics. Secreted proteins FbpA, FbpB and FbpC attach mycolic acids to arabinogalactan, generating mycolic acid methyl esters (MAME) or trehalose, generating α,α′-trehalose dimycolate (TDM; also called cord factor). Our studies of *Mycobacterium smegmatis* showed that:

- disruption of *fbpA* did not affect MAME levels but resulted in a 45% reduction of TDM.
- the *fbpA* mutant displayed increased sensitivity to both front-line tuberculosis-targeted drugs as well as other broad-spectrum antibiotics widely used for antibacterial chemotherapy.
- the irregular, hydrophobic surface of wild-type *M. smegmatis* colonies became hydrophilic and smooth in the mutant.
- while expression of *M. smegmatis fbpA* restored defects of the mutant, heterologous expression of the *Mycobacterium tuberculosis fbpA* gene was less effective.
- a single mutation in the *M. smegmatis* FbpA esterase domain inactivated its ability to provide antibiotic resistance.

To conclude, these data show that production of TDM by FbpA is essential for the intrinsic antibiotic resistance and normal colonial morphology of some mycobacteria and support the concept that FbpA-specific inhibitors, alone or in combination with other antibiotics, could provide an effective treatment for tuberculosis and other mycobacterial diseases.

Part II Here a system is described that coordinates resistance to drugs that have penetrated the envelope, allowing mycobacteria to tolerate diverse classes of antibiotics that inhibit cytoplasmic targets. This system depends on *whiB7,* and its characterisation revealed that:

- *whiB7* is a gene that pathogenic *Mycobacterium* shares with *Streptomyces* (phylogenetically related genus known as the source of diverse antibiotics).
- in *M. tuberculosis, whiB7* is induced by subinhibitory concentrations of antibiotics (erythromycin, tetracycline and streptomycin) and by exposure to fatty acids that pathogenic *Mycobacterium* species may accumulate internally or encounter within eukaryotic hosts during infection.
- *whiB7* null mutants (*Streptomyces* and *Mycobacterium*) are hypersusceptible to antibiotics *in vitro*.
- *M. tuberculosis* is also antibiotic sensitive within a monocyte model system.

– gene expression profiling analyses demonstrate that *whiB7* transcription determines drug resistance by activating expression of a regulon including genes involved in ribosomal protection and antibiotic efflux.

To conclude, components of the *whiB7* system may serve as attractive targets for the identification of inhibitors that render *M. tuberculosis* or multidrug-resistant derivatives more antibiotic-sensitive.

Part III Characterisation of WhiB7 protein (first step for the design of WhiB7 inhibitors) revealed that:

– this transcriptional regulator has (like other members of the WhiB family) a specific tryptophan/glycine-rich region and four conserved cysteine residues; in addition, it has a DNA-binding domain (AT-hook) at its carboxy terminus.
– soluble forms of a number of WhiB family proteins were obtained by overexpression and labelled by the isotopes ^{15}N and ^{13}C for NMR analysis.
– when reconstituted under anaerobic, reducing conditions by iron(II) and sulphide, all proteins formed a complex with an oxygen-sensitive iron-sulphur cluster (FeS cluster).
– the NMR spectra were obtained showing that these proteins were largely unstructured both in apo and reconstituted forms.

To conclude, WhiB-like proteins, without a further binding partner, are in solution largely unstructured both in their apo and reconstituted form with a FeS cluster.

Publications of the NRP 49 project

Nguyen LS, Jensen PR, et al.
WhiB7 dependent multidrug resistance in *Mycobacterium*.
Eur J. Biochem. In press 2007.

Nguyen L, Thompson CJ.
Foundations of antibiotic resistance in bacterial physiology: the mycobacterial paradigm.
Trends Microbiol. 2006 Jul;14(7):304-12. Epub 2006 Jun 8.

Nguyen L, Chinnapapagari S, Thompson CJ.
FbpA-dependent biosynthesis of trehalose dimycolate is required for the intrinsic multidrug resistance, cell wall structure, and colonial morphology of *Mycobacterium smegmatis*.
J Bacteriol. 2005 Oct;187(19):6603-11.

Morris RP, Nguyen L, Gatfield J, Visconti K, Nguyen K, Schnappinger D, Ehrt S, Liu Y, Heifets L, Pieters J, Schoolnik G, Thompson CJ.
Ancestral antibiotic resistance in *Mycobacterium tuberculosis*.
Proc Natl Acad Sci U S A. 2005 Aug 23;102(34):12200-5. Epub 2005 Aug 15

back to the NRP 49 final report