OVERVIEW**Mycobacteria research in the post-genomic era**

Douglas B. Young

Tel: +44 20 7594 3962. Fax: +44 20 7594 3095. e-mail: d.young@ic.ac.uk

Centre for Molecular Microbiology and Infection, Department of Infectious Diseases and Microbiology, Faculty of Medicine, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK

Keywords: cell wall, lipids, drug targets, immune response.

Background

The last decade has witnessed renewed concern about the impact of tuberculosis on global health against a background of synergy with HIV, the emergence of drug-resistant strains and well-publicized outbreaks in the USA and Europe. In contrast, successful implementation of multidrug therapy has fostered complacency about leprosy as a threat to public health, even though the number of new cases remains disturbingly high in the most prevalent areas (Lockwood, 2002). Interest has been maintained in the opportunistic 'atypical' mycobacteria, in relation to their ability to cause disease in immunocompromised individuals and, increasingly, their possible role in modifying immune responses to the major pathogenic species (Brandt *et al.*, 2002). Rapid-growing non-pathogenic mycobacteria have had an important role as model organisms in the study of fundamental aspects of microbiology.

Over the same period, there has been a renaissance in microbiological interest in the mycobacterial genus with the introduction of new molecular genetic tools. The slow growth rate and rugged cell wall structure make mycobacteria – particularly the major pathogens – difficult targets for classical genetics and biochemistry. These barriers are substantially overcome by approaches based on dissection of mycobacteria at the level of DNA, however, and the present special issue of *Microbiology* celebrates the wealth of current interest in research on the mycobacterial genus in the post-genomic era.

The mycobacteria special issue

Much of modern mycobacteriology is conducted within the framework provided by the genome sequence, and the special issue is introduced by a review article from Cole that presents a definitive description of the application of genomics to our current understanding of the *M. tuberculosis* complex (Cole, 2002). A useful update on the genome annotation of *M. tuberculosis* H37Rv is provided in a paper by Camus *et al.* (2002). Genome comparison amongst different isolates and

between species plays an important role in understanding the epidemiology and evolution of the mycobacteria, and papers by Dullaghan and by Howard contribute to this area, describing a novel two-dimensional electrophoresis genotyping system (Dullaghan *et al.*, 2002) and a mobile genetic element from *Mycobacterium abscessus*, a rapid-growing opportunistic pathogen (Howard *et al.*, 2002). There is clearly important scope for linking this genotypic diversity to phenotypic variations such as the differences in cell wall mycolic acid structures used by Astola *et al.* (2002) to discriminate between strains of *Mycobacterium goodii*.

Several papers take up the challenge of moving from genome sequence to functional biology. McAdam *et al.* (2002) describe mapping of insertion points in a transposon library of *Mycobacterium tuberculosis*, identifying mutations affecting more than 350 individual ORFs. In addition to providing 'off-the-shelf' mutants available for researchers with an interest in these particular genes, this study begins to define the set of *M. tuberculosis* genes that are dispensable for growth in laboratory media. An important aspect of the study is that it highlights a bias in the pattern of Tn5370 insertion events, with predominant targeting of regions with lower than average G + C content. Mutagenesis by allele replacement is illustrated in two papers. Bardarov *et al.* (2002) describe a novel technique for the introduction of targeted mutations using a temperature-sensitive transducing phage, while Parish & Stoker (2002) focus on analysis of genes that are essential for *in vitro* growth, specifically those involved in the chorismate pathway for aromatic amino acid biosynthesis.

There is considerable interest in understanding mechanisms involved in the regulation of gene expression in mycobacteria. Santangelo *et al.* (2002) have analysed the expression of genes implicated in entry into mammalian host cells (the *mce* operon) and report on a novel transcriptional regulator. Stewart and colleagues describe the use of microarray-based whole genome expression profiling to explore the adaptation of *M. tuberculosis* to environmental changes. Focusing on the response to heat shock, they have identified a series

of overlapping regulatory circuits involving alternative sigma factors and two heat shock-specific transcriptional repressors (Stewart *et al.*, 2002). As in the case of the oxidative response genes described by Master *et al.* (2002), stress-induced changes in mycobacteria are likely to be important in survival during infection and as signals for the host immune defence (Stewart *et al.*, 2001).

Analysis of the structure of mycobacterial cell wall components has provided a consistent topic for outstanding biological chemistry; increasingly this is achieved in tandem with a molecular genetic approach. Kremer *et al.* (2002) demonstrate that, in addition to the heat-shock proteins discussed above, changes in cell wall lipids are also a feature of the adaptive response to temperature stress. It is probable that remodelling of the cell wall will be an important component in the physiology of mycobacterial infection, and Ohja *et al.* (2002) describe a novel polar glycopeptidolipid synthesized by *M. smegmatis* grown in glucose-limited culture. Papers from Etienne *et al.* and from Jeevarajah *et al.* make use of molecular genetic tools to study the biosynthesis of glycopeptidolipids in *Mycobacterium smegmatis* (Jeevarajah *et al.*, 2002), and to demonstrate the important role that they play in determining surface properties and permeability (Etienne *et al.*, 2002). Hancock *et al.* (2002) provide further information about the assembly of arabinogalactan and peptidoglycan, a central step in construction of the mycobacterial cell wall. Two papers report investigation of lipoarabinomannan, a cell wall component shown to be crucial in triggering responses in the infected host. Kaur *et al.* (2002) provide a detailed description of arabinose motifs involved in antibody binding to lipoarabinomannan and arabinogalactan, and Ludwiczak and colleagues report an alteration in the fine structure of lipoarabinomannan in a *phoP* knock out mutant of *M. tuberculosis* (Ludwiczak *et al.*, 2002). The *phoP* gene encodes part of a two-component response regulator and the knock out strain is attenuated in a mouse model (Perez *et al.*, 2001). It is not clear whether the altered lipoarabinomannan structure is a direct or a secondary effect of the mutation. Garton and colleagues report that lipids are involved not only in the cell wall structure, but also in intracellular storage depots. They observed lipophilic inclusions in *M. smegmatis* cultures grown *in vitro*, and also in *M. tuberculosis* in sputum (Garton *et al.*, 2002), adding to the growing body of evidence that lipids are an essential source of nutrition for *M. tuberculosis* within the infected host (McKinney *et al.*, 2000).

An important goal of current research is to identify targets for new drugs. McLean *et al.* (2002) describe the potential for exploitation of the multiple cytochrome P450 homologues in *M. tuberculosis* as drug targets, and Douglas *et al.* (2002) report on promising antimycobacterial activity of thiolactomycin derivatives. Turner and colleagues used molecular genetic tools to construct luminescent reporter strains of mycobacteria suitable for rapid assessment of drug action. Carrying out experiments with infected tissues, they stress the im-

portance of interactions between drugs and host immunity, highlighting synergy between pyrazinamide and early events in the cell-mediated immune response (Turner *et al.*, 2002).

Finally, a series of papers report on the immune response to mycobacterial infection. Collins *et al.* (2002) describe the use of mycobacterial mutants for vaccine development, while Gomes & Appelberg (2002) have used knock out mice to demonstrate that reactive oxygen species are not required for resistance to *Mycobacterium avium* infection. Fattorini *et al.* (2002) report that low dose infection with *M. avium* has a protective effect against subsequent high dose challenge, and Gruppo *et al.* (2002) and DesJardin *et al.* (2002) highlight the important role played by cell surface adhesion molecules during *M. tuberculosis* infection in mice and in human macrophages.

Conclusion

This special issue provides an impressive demonstration of the very active current research on the molecular microbiology of the mycobacteria. Will this be sufficient to reverse the progressive rise in global tuberculosis? The combined advances in molecular genetics and cell wall biochemistry provide a scientific platform suitable for development of a new generation of drugs directed against the emerging multidrug-resistant strains. As with existing drugs, it is likely that these will act primarily against actively dividing organisms, and that prolonged treatment regimens will be required for a reliable cure. Prospects for the development of shorter treatments and for the rational design of effective vaccines will depend on our ability to translate successful studies of mycobacteria in the test tube and in experimental models into an improved understanding of the complex biology of mycobacteria during their prolonged residence in human tissues.

References

- Astola, J., Muñoz, M., Sempere, M., Coll, P., Luquin, M. & Valero-Guillen, P. L. (2002). The HPLC-double-cluster pattern of some *Mycobacterium gordonae* strains is due to their dicarboxymycolate content. *Microbiology* **148**, 3119–3127.
- Bardarov, S., Bardarov, S., Jr, Pavelka, M. S., Jr, Sambandamurthy, V., Larsen, M., Tufariello, J., Chan, J., Hatfull, G. & Jacobs, W., Jr (2002). Specialized transduction: an efficient method for generating marked and unmarked targeted gene disruptions in *Mycobacterium tuberculosis*, *M. bovis* BCG and *M. smegmatis*. *Microbiology* **148**, 3007–3017.
- Brandt, L., Feino Cunha, J., Weinreich Olsen, A., Chilima, B., Hirsch, P., Appelberg, R. & Andersen, P. (2002). Failure of the *Mycobacterium bovis* BCG vaccine: some species of environmental mycobacteria block multiplication of BCG and induction of protective immunity to tuberculosis. *Infect Immun* **70**, 672–678.
- Camus, J.-C., Pryor, M. J., Médigue, C. & Cole, S. T. (2002). Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv. *Microbiology* **148**, 2967–2973.
- Cole, S. T. (2002). Comparative and functional genomics of the *Mycobacterium tuberculosis* complex. *Microbiology* **148**, 2919–2928.

- Collins, D. M., Wilson, T., Campbell, S., Buddle, B. M., Wards, B. J., Hotter, G. & de Lisle, G. W. (2002). Production of avirulent mutants of *Mycobacterium bovis* with vaccine properties by the use of illegitimate recombination and screening of stationary-phase cultures. *Microbiology* **148**, 3019–3027.
- Desjardin, L. E., Kaufman, T. M., Potts, B., Kutzbach, B., Yi, H. & Schlesinger, L. S. (2002). *Mycobacterium tuberculosis*-infected human macrophages exhibit enhanced cellular adhesion with increased expression of LFA-1 and ICAM-1 and reduced expression and/or function of complement receptors, FcγRII and the mannose receptor. *Microbiology* **148**, 3161–3171.
- Douglas, J. D., Senior, S. J., Morehouse, C., Phetsukiri, B., Campbell, I. B., Besra, G. S. & Minnikin, D. E. (2002). Analogues of thiolactomycin: potential drugs with enhanced anti-mycobacterial activity. *Microbiology* **148**, 3101–3109.
- Dullaghan, E. M., Malloff, C. A., Li, A. H., Lam, W. L. & Stokes, R. W. (2002). Two-dimensional bacterial genome display: a method for the genomic analysis of mycobacteria. *Microbiology* **148**, 3111–3117.
- Etienne, G., Villeneuve, C., Billman-Jacobe, H., Astarie-Dequeker, C., Dupont, M.-A. & Daffé, M. (2002). The impact of the absence of glycopeptidolipids on the ultrastructure, cell surface and cell wall properties, and phagocytosis of *Mycobacterium smegmatis*. *Microbiology* **148**, 3089–3100.
- Fattorini, L., Nisini, R., Fan, Y., Li, Y.-J., Tan, D., Mariotti, S., Teloni, R., Iona, E. & Orefici, G. (2002). Exposure of BALB/c mice to low doses of *Mycobacterium avium* increases resistance to a subsequent high-dose infection. *Microbiology* **148**, 3173–3181.
- Garton, N. J., Christensen, H., Minnikin, D. E., Agedbola, R. A. & Barer, M. R. (2002). Intracellular lipophilic inclusions of mycobacteria *in vitro* and in sputum. *Microbiology* **148**, 2951–2958.
- Gomes, M. S. & Appelberg, R. (2002). NRAMP1- or cytokine-induced bacteriostasis of *Mycobacterium avium* by mouse macrophages is independent of the respiratory burst. *Microbiology* **148**, 3155–3160.
- Gruppo, V., Turner, O. C., Orme, I. M. & Turner, J. (2002). Reduced up-regulation of memory and adhesion/integrin molecules in susceptible mice and poor expression of immunity to pulmonary tuberculosis. *Microbiology* **148**, 2959–2966.
- Hancock, I. C., Carman, S., Besra, G. S., Brennan, P. J. & Waite, E. (2002). Ligation of arabinogalactan to peptidoglycan in the cell wall of *Mycobacterium smegmatis* requires concomitant synthesis of the two wall polymers. *Microbiology* **148**, 3059–3067.
- Howard, S. T., Byrd, T. F. & Lyons, C. R. (2002). A polymorphic region in *Mycobacterium abscessus* contains a novel insertion sequence element. *Microbiology* **148**, 2987–2996.
- Jeevarajah, D., Patterson, J. H., McConville, M. J. & Billman-Jacobe, H. (2002). Modification of glycopeptidolipids by an O-methyltransferase of *Mycobacterium smegmatis*. *Microbiology* **148**, 3079–3087.
- Kaur, D., Lowary, T. L., Vissa, V. D., Crick, D. C. & Brennan, P. J. (2002). Characterization of the epitope of anti-lipoarabinomannan antibodies as the terminal hexaarabinofuranosyl motif of mycobacterial arabinans. *Microbiology* **148**, 3049–3057.
- Kremer, L., Guérardel, Y., Gurcha, S. S., Loch, C. & Besra, G. S. (2002). Temperature-induced changes in the cell-wall components of *Mycobacterium thermoresistibile*. *Microbiology* **148**, 3145–3154.
- Lockwood, D. N. J. (2002). Leprosy elimination – a virtual phenomenon or a reality? *BMJ* **324**, 1516–1518.
- Ludwiczak, P., Gilleron, M., Bordas, Y., Martin, C., Gicquel, B. & Puzo, G. (2002). *Mycobacterium tuberculosis phoP* mutant: lipoarabinomannan molecular structure. *Microbiology* **148**, 3029–3037.
- Master, S. S., Springer, B., Sander, P., Boettger, E. C., Deretic, V. & Timmins, G. S. (2002). Oxidative stress response genes in *Mycobacterium tuberculosis*: role of *ahpC* in resistance to peroxynitrite and stage-specific survival in macrophages. *Microbiology* **148**, 3139–3144.
- McAdam, R. A., Quan, S., Smith, D. A. & 11 other authors (2002). Characterization of a *Mycobacterium tuberculosis* H37Rv transposon library reveals insertions in 351 ORFs and mutants with altered virulence. *Microbiology* **148**, 2975–2986.
- McKinney, J. D., Honer zu Bentrup, K., Munoz-Elias, E. J. & 7 other authors (2000). Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* **406**, 735–738.
- McLean, K. J., Marshall, K. R., Richmond, A., Hunter, I. S., Fowler, K., Kieser, T., Gurcha, S. S., Besra, G. S. & Munro, A. W. (2002). Azole antifungals are potent inhibitors of cytochrome P450 mono-oxygenases and bacterial growth in mycobacteria and streptomycetes. *Microbiology* **148**, 2937–2949.
- Ojha, A. Kr., Varma, S. & Chatterji, D. (2002). Synthesis of an unusual polar glycopeptidolipid in glucose-limited culture of *Mycobacterium smegmatis*. *Microbiology* **148**, 3039–3048.
- Parish, T. & Stoker, N. G. (2002). The common aromatic amino acid biosynthesis pathway is essential in *Mycobacterium tuberculosis*. *Microbiology* **148**, 3069–3077.
- Perez, E., Samper, S., Bordas, Y., Guilhot, C., Gicquel, B. & Martin, C. (2001). An essential role for *phoP* in *Mycobacterium tuberculosis* virulence. *Mol Microbiol* **41**, 179–187.
- Santangelo, M. P., Goldstein, J., Alito, A. & 7 other authors (2002). Negative transcriptional regulation of the *mce3* operon in *Mycobacterium tuberculosis*. *Microbiology* **148**, 2997–3006.
- Stewart, G. R., Snewin, V. A., Walzl, G. & 7 other authors (2001). Overexpression of heat shock proteins reduces survival of *Mycobacterium tuberculosis* in the chronic phase of infection. *Nat Med* **7**, 732–737.
- Stewart, G. R., Wernisch, L., Stabler, R., Mangan, J. A., Hinds, J., Laing, K. G., Young, D. B. & Butcher, P. D. (2002). Dissection of the heat-shock response in *Mycobacterium tuberculosis* using mutants and microarrays. *Microbiology* **148**, 3129–3138.
- Turner, D. J., Hoyle, S. L., Snewin, V. A., Gares, M.-P., Brown, I. N. & Young, D. B. (2002). An *ex vivo* culture model for screening drug activity against *in vivo* phenotypes of *Mycobacterium tuberculosis*. *Microbiology* **148**, 2929–2936.