Genomics and the chemotherapy of leprosy

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Summary The information deduced from the genome sequence of Mycobacterium leprae is of immense value for the chemotherapy of leprosy. Knowing the complete set of genes, enzymes and proteins allows us to understand why some drugs are without effect whereas others are fully active. It may also enable better use to be made of existing drugs, such as β-lactams, and opens new avenues for the development of novel compounds. M. leprae is relatively susceptible to a wide range of drugs, unlike the highly related tubercle bacillus, and several new multidrug regimens are in clinical trials. Genomics provides a number of possible explanations for this broader susceptibility as some of the genes encoding enzymes involved in antibiotic inactivation have decayed whereas the number of transporters available to contribute to drug efflux is considerably lower than in Mycobacterium tuberculosis. Several leads for new drug targets have been uncovered.

Introduction

The WHO-recommended multidrug therapy (MDT) for leprosy has been, without question, one of the major success stories in the field of public health. There is, however, no room for complacency as the incidence of detected cases of leprosy has not fallen during the last decade and the spectre of drug resistance is never far away. This has been well illustrated by the increased spread of multidrug resistant tuberculosis during the last decade. Furthermore, regimens can always be improved by increasing efficacy or reducing duration and this is only likely to be achieved by employing drugs that are stronger or more effective than dapsone and clofazimine.

A number of compounds, such as minocycline, various fluoroquinolones and macrolides, have shown excellent activity in the mouse model of leprosy, and in limited clinical trials, and ROM, a new regimen for the treatment of single lesion paucibacillary leprosy, comprising rifampicin, ofloxacin and minocycline, has shown particular promise. In contrast to the situation in tuberculosis, where new chemotherapeutic agents are desperately

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needed, the future of leprosy treatment is well poised. Nevertheless, it is important not to neglect any opportunities for improvement and to remain aware of potential new leads for strengthening the chemotherapy of leprosy.

Drug resistance in bacteria can result from four different mechanisms. The commonest one, found in bacteria, is the enzymatic inactivation of the antibiotic by modification or hydrolysis. Since the genes encoding these enzymes are generally transferable, this resistance mechanism has often limited the usefulness of numerous β-lactam and aminoglycoside compounds. Transferable drug resistance has not been reported among the intracellular mycobacteria where the most frequent mechanism encountered is alteration of the drug target by mutation.9,10 which is the case for rifampicin resistance in *Mycobacterium leprae*.11,12 In some pathogens, innate drug resistance results from efficient permeability barriers preventing the entry of sufficient concentrations of drugs into the cell, and this appears to be operational in some mycobacteria.13,14 Active efflux of antibiotics also occurs from various pathogens, including mycobacteria,15 and is mediated by protein pumps that belong to different families such as the major facilitator superfamily (MFS), the resistance-nodulation-division (RND) superfamily, the ATP-binding cassette (ABC) superfamily, or the small multidrug resistance (SMR) superfamily.16-18

The genome sequence of *M. leprae*19 provides clear explanations for the natural resistance of the leprosy bacillus to many antitubercular compounds and also indicates areas of potential susceptibility where existing drugs might find application. Our aim here is to discuss these topics in the light of the available clinical and microbiological knowledge.

Materials and methods

Bioinformatic analysis was performed using the procedures outlined previously19,20 in conjunction with the relational databases, Leproma (http://genolist.pasteur.fr/Leproma/) and Tuberculist (http://genolist.pasteur.fr/Tuberculist/).

Results and discussion

**RIFAMPICIN, DAPSONE AND CLOFAZIMINE**

WHO-recommended MDT relies on the association of three agents: rifampicin, dapsone and clofazimine. Since rifampicin is the backbone of leprosy treatment, the genome sequencing project began by studying the region encompassing the *rpoB* gene, encoding the drug target, the β-subunit of DNA-dependent RNA polymerase.22 The sequence information, in conjunction with a panel of well-characterized resistant patient isolates,23 enabled the molecular basis of rifampicin resistance to be elucidated.11 Missense mutations were found in a restricted region of *rpoB* that probably reduces the affinity of the drug for RNA polymerase, and this information has been used to develop rapid diagnostic tests for resistance.12,24,25 There is only one *rpoB* and no other genes associated with rifampicin resistance, like those mediating its ribosylative inactivation in certain actinomycetes,26,27 could be found in the genome. This explains why other rifamycin derivatives that have the same drug target as rifampicin are inactive because of cross-resistance, and cannot be used as replacement drugs in the case of rifampicin resistance. However, some of them have pharmacokinetic
parameters more favourable than those of rifampicin for monthly administration. For example, rifapentine, a long lasting rifamycin derivative, has a serum half life 6 times longer than that of rifampicin and gives in humans an AUC after oral administration of 600 mg, 3 times larger than that given by 600 mg of rifampicin. Consequently, a monthly regimen containing rifapentine combined with a new fluoroquinolone and minocycline is under intensive investigation at the present time.67

Possibly as a result of its widespread, initial use in monotherapy, primary resistance to dapsone became common in the 1960s. Again, genomics was of great importance for understanding the basis of this resistance as the availability of the folate synthase gene sequence, \textit{folP}1, enabled Kai and coworkers to demonstrate the presence of mutations in the gene from dapsone resistant strains.26 Intriguingly, both \textit{Mycobacterium tuberculosis} and \textit{M. leprae} have a second gene, \textit{folP}2, that resembles \textit{folP}1, but this does not encode folate synthase nor mediate dapsone sensitivity.77 Since folate synthesis is an essential activity, missense mutations occur that lead to amino acid substitutions at positions 53 and 55,29,29 and from the crystal structure of the \textit{M. tuberculosis} enzyme it is apparent that these are located in loop 2 near the active site.30 The availability of the three-dimensional structure will allow lead compounds for antimycobacterial drug design to be designed in a rational manner, and clearly, if successful, this could benefit leprosy treatment.

The third component of MDT, clofazirinine, is the least well understood in terms of its mode of action and resistance mechanism.31 Its activity is primarily confined to mycobacteria where it is believed to bind to DNA. The genome sequence has yet to provide clues to possible targets.

\textbf{ISONIAZID, ETHIONAMIDE, CELL WALL INHIBITORS}

The unusual cell wall of mycobacteria has provided a rich and specific source of drug targets and, with the exception of rifampicin, three of the four agents used in the short course chemotheraphy of tuberculosis block the synthesis of cell wall components. Somewhat surprisingly, in light of the conserved, if somewhat simplified nature of the cell wall of \textit{M. leprae}, none of these compounds is active, but explanations for their lack of efficacy are provided by the genome sequence. Isoniazid (INH) is exquisitely potent on the tubercle bacillus, where it is converted by catalase-peroxidase,33 to an iso-nicotinoyl radical which forms an adduct with NADH that blocks the action of the enoyl-ACP reductase, InhA.35,36 This enzyme is involved in mycolic acid synthesis, as part of the fatty acid synthase II complex (FAS II), and is also a target for ethionamide.37 In addition, INH interacts directly with another FAS II component, the beta-ketoacyl-ACP synthase, KasA.38,39 Although \textit{M. leprae} has an intact FAS II system and functional \textit{kasA} and \textit{inhA} genes, it is not susceptible to clinically significant levels of INH as a result of multiple lesions in the catalase-peroxidase (\textit{katG}) gene.40,41 Unlike some other mycobacteria,42 the genome of \textit{M. leprae} does not contain a second catalase-peroxidase gene.43 By contrast, as the FAS II complex is active, it is possible that \textit{M. leprae} will be susceptible to triclosan, which inhibits InhA, and to an old compound, thioclocamycin, which affects KasA in \textit{M. tuberculosis}, and other bacteria.35,36 The leprosy bacillus is known to be inhibited by prothionamide (combined with isoniazid and dapsone in the fixed drug combination isoprodian), a hepatotoxic drug which, like ethionamide, should target InhA. It has recently been shown that ethionamide requires an activation step that is catalysed by a mono-oxygenase belonging to a 14-membered family (http://genolist.pasteur.fr/Tuberculist/mast/
It is of some interest that expression of the mono-oxygenase gene, \textit{Rv3854c} (\textit{ethA} or \textit{etaA}) is regulated by a TetR repressor protein encoded by the neighbouring gene \textit{Rv3855} (\textit{ethR}, \textit{etaR}). Both the monoxygenase and the regulatory gene have been conserved in \textit{M. leprae}, (ML0064, ML0065), implying that thioamide activation should proceed in a similar manner. Astonishingly, the monoxygenase gene is the sole survivor of the P14.2 family and, in the face of such extensive gene loss, this implies that its physiological function must be important for \textit{M. leprae} in particular, and mycobacteria in general.

Ethambutol inhibits the arabinosyltransferase(s), membrane-bound enzymes involved in the polymerization of arabinan which, in turn, impacts on arabinogalactan and lipooligosaccharide production thereby destabilizing the cell wall.\textsuperscript{19} Using complementary approaches with \textit{M. tuberculosis} and \textit{Mycobacterium avium},\textsuperscript{46,47} these enzymes were shown to be encoded by linked genes, \textit{emb(C)AB}, that have evolved by a gene duplication mechanism and are probably controlled by the regulatory gene, \textit{embR}. While \textit{M. leprae} has the \textit{embAB} operon it appears to have lost \textit{embR}, which may mean that arabinosyltransferase production is no longer regulated.\textsuperscript{19} Missense mutations located in a tetrapeptide at positions 303–306 of \textit{EmbB} are responsible for acquired drug resistance in the majority of clinical isolates of \textit{M. tuberculosis}\textsuperscript{48} and in laboratory mutants of \textit{Mycobacterium smegmatis}.\textsuperscript{49} The \textit{embB} gene of \textit{M. leprae} harbours a ‘mutation’ at this position\textsuperscript{46} and this undoubtedly accounts for its natural resistance to ethambutol.

Pyrazinamide is the third antitubercular agent that is believed to affect cell wall biogenesis possibly through indirect effects on fatty acid synthesis mediated by FAS I in the tubercle bacillus.\textsuperscript{50} Drug activity requires its conversion to pyrazinoic acid in a reaction catalysed by the pyrazinamidase enzyme encoded by \textit{pncA} in \textit{M. tuberculosis}\textsuperscript{51} or by a broad-spectrum amidase coded for by \textit{pzaA} in \textit{M. smegmatis}.\textsuperscript{52,53} Resistance is associated with mutations that inactivate \textit{pncA}, or alter the stability of pyrazinamidase, and in some mycobacteria, pyrazinoic acid efflux systems may also contribute.\textsuperscript{54,55} The \textit{pncA} gene of \textit{M. leprae} has been inactivated and the genome contains no counterpart of \textit{pzaA}. Pyrazinamide is therefore likely to be of no therapeutic value for leprosy.

\textbf{TRANSLATION INHIBITORS}

Antibiotics belonging to the tetracycline, aminoglycoside and macrolide families are potent inhibitors of protein synthesis. The aminoglycosides streptomycin and kanamycin show strong bactericidal activity in the mouse, and streptomycin has been used to treat leprosy in humans.\textsuperscript{56} Resistance to streptomycin in mycobacteria arises as a result of missense mutations to the \textit{rpsL} and \textit{rrs} genes encoding the drug targets, the ribosomal protein S12 and the 16S rRNA, respectively.\textsuperscript{57,58} Likewise, resistance to kanamycin is due to base changes around position 1400 in the 16S rRNA of \textit{M. tuberculosis} that prevent the drug from binding.\textsuperscript{59} As expected, both genes have wild type sequences in \textit{M. leprae} thereby explaining its susceptibility to streptomycin and kanamycin.

Clarithromycin is a macrolide antibiotic that shows bactericidal activity against \textit{M. leprae} in mice and humans.\textsuperscript{5,62} Susceptibility can be attributed to the wild type sequence of the \textit{rrl} gene, encoding the 23S rRNA. A to G transitions affecting positions 2058 and 2059 of this RNA have been described in clarithromycin resistant strains of \textit{M. avium} and \textit{Mycobacterium kansasii}, among others.\textsuperscript{50,63} Minocycline, a second-generation tetracycline, is also active on \textit{M. leprae},\textsuperscript{5,62} probably as a result of its ability to bind to a site on the ribosome comprising
proteins S7, S14, S19 and the 3' domain of 16S rRNA. At present nothing is known about minocycline resistance in mycobacteria, although tetracycline resistance has been studied intensively in other Gram positive bacteria where it often involves efflux or ribosome protection systems.

Fusidic acid is another broad spectrum antibiotic that targets the ribosome and inhibits the growth of M. leprae. It acts by preventing release of elongation factor EF-G from the ribosome and, in enteric bacteria, fusidic acid resistance is due to missense mutations in three highly conserved regions of the efg gene. The availability of the efg sequence of M. leprae allows genotypic tests for resistance to be developed should the need arise.

QUINOLONES

Fluoroquinolones offer great potential to the future therapy of leprosy and have shown outstanding activity in vitro and in vivo. Ofloxacin is a key component of the ROM regimen, discussed above, although some of the newer fluoroquinolones such as moxifloxacin appear to be even more bactericidal. A limited number of cases of fluoroquinolone resistance have been reported and, as in M. tuberculosis, these involve amino acid substitutions in the quinolone resistance determining region, QRDR, of the DNA gyrase A protein. In some bacteria, resistance also results from alterations of DNA topoisomerase IV, but genomics suggests that both M. leprae and M. tuberculosis lack this function. The QRDR is situated very near the active site of GyrA and most unusually this region of gyrA has acquired an intein sequence coding for a putative homing endonuclease. In consequence, production of active GyrA requires excision of the intein from the nascent polypeptide by protein splicing. There are three other inteins in the ML0593, dnaB, and recA genes of M. leprae, although these all differ in size and sequence. Since both gyrA and dnaB encode essential functions they are valid drug targets whose functions would be lost if protein splicing were blocked.

ANTIBIOTIC INACTIVATION

Of the several reasons why M. leprae shows susceptibility to a broader range of drugs than M. tuberculosis, one is provided by the finding that its genome contains far fewer genes encoding enzymes that could inactivate or modify antibiotics. Only two of the 10 genes, annotated as being putatively involved in antibiotic modification in M. tuberculosis, are predicted to be functional in M. leprae. One of these, ML2551, encodes an aminoglycoside-2'-N-acetyltransferase that may be involved in peptidoglycan modification.

While the tubercle bacillus has seven known or potential β-lactamase genes (Table 1), M. leprae has only two that appear to be functional (ML0270, ML1923). Consequently, β-lactam antibiotics may be more active in leprosy than in tuberculosis. It should be noted that there is no blac ortholog encoding the class-A β-lactamase present in M. tuberculosis which contributes to its innate resistance to β-lactam antibiotics. As expected of a class-A enzyme, inhibition was achieved by the β-lactamase inhibitors clavulanate or sulbactam, thereby raising the possibility of treating tuberculosis with penicillins and cephalosporins in conjunction with such inhibitors. There has been some recent interest in using these combinations to treat leprosy as well, inspired in part by Shephard’s observations that, of the 12 β-lactams tested, two cephalosporins and one cephamycin were active in the mouse
Table 1. Predicted β-lactamase-like proteins in tuberculosis and leprosy bacilli

<table>
<thead>
<tr>
<th>Gene</th>
<th>Predicted function in M. tuberculosis</th>
<th>M. leprae*</th>
</tr>
</thead>
<tbody>
<tr>
<td>blac (Rv2068c)</td>
<td>Class A β-lactamase</td>
<td>del</td>
</tr>
<tr>
<td>lipD (Rv1923)</td>
<td>Similar to esterase, β-lactamase</td>
<td>del</td>
</tr>
<tr>
<td>lipW (Rv2905)</td>
<td>Lipoprotein with slight similarity to β-lactamase</td>
<td>ML1644, ps</td>
</tr>
<tr>
<td>lipF (Rv3059)</td>
<td>Lipoprotein with slight similarity to class C β-lactamase</td>
<td>ML1923</td>
</tr>
<tr>
<td>Rv0406c</td>
<td>β-lactamase-like protein with Pfam match PF00753</td>
<td>ML0270</td>
</tr>
<tr>
<td>Rv0007</td>
<td>Similar to PBP 4, class C β-lactamase</td>
<td>ML2116, ps</td>
</tr>
<tr>
<td>Rv1913</td>
<td>Similar to dehydrase, metallo-β-lactamase</td>
<td>ML2061, ps</td>
</tr>
</tbody>
</table>

*ps, denotes pseudogene; del, missing probably deleted.

Footpad model. In two studies involving β-lactam antibiotics with β-lactamase inhibitors, bactericidal activity was reported.

Drug Efflux

Another potential mechanism that could contribute to natural antimicrobial resistance is drug efflux and, in some pathogenic bacteria, this is known to be mediated by transmembrane proteins belonging to the ATP-binding cassette (ABC), and major facilitator superfamilies (MFS), the small multidrug resistance family (SMR) and the resistance/nodulation/division family (RND). M. leprae has proteins belonging to all four of these families but they are considerably less abundant than in the tubercle bacillus.

Careful analysis of the ABC transport proteins of M. tuberculosis has been undertaken, and these can be divided into import and export systems on the basis of their structure and organization. Of the 11 potential drug export systems predicted (Table 2), only six remain in M. leprae. Pseudogenes for two may be found and the remainder appear to have been deleted. The drrABC system is very similar to those produced by various Streptomyces spp. and like them may also be involved in the export of daunorubicin-like molecules. Investigations into the possible contribution of some of the ~30 MFS proteins of M. tuberculosis to drug efflux have been reported and these can be used to interpret the likely role of the few remaining orthologs in M. leprae. Two MFS proteins, Rv1258c and Rv1410c, have been shown to serve as proton motive force-dependent drug pumps that confer increased resistance to several aminoglycosides and tetracycline when expressed in M. smegmatis. Both of these functions have been conserved in M. leprae, together with a third MFS protein, EfpA (Table 2) which is similar to the multidrug resistance pump, PscA. Recently, expression of EfpA has been shown to be strongly induced during drug-mediated inhibition of cell wall synthesis in M. tuberculosis. It is possible that these three conserved MFS proteins also act as drug pumps in M. leprae (Table 2).

M. leprae, like the tubercle bacillus, has only one member of the SMR family, the 108 residue ML1756 protein (Table 2, equivalent to Rv3065) and this has four transmembrane stretches like its relatives. When expressed in M. smegmatis the Rv3065 gene confers resistance to a variety of compounds, including acriflavin, erythromycin, ethidium bromide, safranin O, and pyronin Y 8586. M. tuberculosis is somewhat unusual as its genome contains 16 genes (Table 2) encoding members of the RND superfamily, an exceptionally high
Table 2. Predicted drug efflux systems in tubercle and leprosy bacilli

<table>
<thead>
<tr>
<th>ABC systems</th>
<th>Genes</th>
<th>Predicted function in M. tuberculosis</th>
<th>M. leprae*</th>
</tr>
</thead>
<tbody>
<tr>
<td>drrABC</td>
<td>Rv1456-36c</td>
<td>Diamorubicin resistance</td>
<td>drrABC</td>
</tr>
<tr>
<td></td>
<td>Rv2586-34c</td>
<td>Antibiotic resistance</td>
<td>ML0590, ML0589</td>
</tr>
<tr>
<td></td>
<td>Rv1237-39c</td>
<td>Antibiotic resistance</td>
<td>ML1033-35, ps</td>
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<tr>
<td></td>
<td>Rv1372-33c</td>
<td>Multidrug resistance</td>
<td>ML1113-14</td>
</tr>
<tr>
<td></td>
<td>Rv1348-49</td>
<td>Multidrug resistance</td>
<td>del</td>
</tr>
<tr>
<td></td>
<td>Rv1194</td>
<td>Multidrug resistance</td>
<td>del</td>
</tr>
<tr>
<td></td>
<td>Rv1190c</td>
<td>Multidrug resistance</td>
<td>ML2084</td>
</tr>
<tr>
<td></td>
<td>Rv1473</td>
<td>Macrolide resistance</td>
<td>ML1816</td>
</tr>
<tr>
<td></td>
<td>Rv1477c</td>
<td>Macrolide resistance</td>
<td>ML1248</td>
</tr>
<tr>
<td></td>
<td>Rv1667-69c</td>
<td>Macrolide resistance</td>
<td>ML1239-40, ps</td>
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<table>
<thead>
<tr>
<th>MFS systems</th>
<th>Genes</th>
<th>Observed or predicted function in M. tuberculosis</th>
<th>M. leprae</th>
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</thead>
<tbody>
<tr>
<td>tap</td>
<td>Rv1258c</td>
<td>Aminoglycoside, tetracycline efflux</td>
<td>ML1104</td>
</tr>
<tr>
<td></td>
<td>Rv2846c</td>
<td>Pseudomonas, tetracycline efflux</td>
<td>ML1362</td>
</tr>
<tr>
<td></td>
<td>Rv1410c</td>
<td>Probable drug efflux protein</td>
<td>ML0556</td>
</tr>
<tr>
<td></td>
<td>Rv1877, Rv2044, Rv2335c</td>
<td>Probable drug efflux protein</td>
<td>All missing</td>
</tr>
<tr>
<td></td>
<td>Rv3243, Rv3728, Rv3239c</td>
<td>Probable drug efflux protein</td>
<td>All missing</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>SMR systems</th>
<th>Genes</th>
<th>Predicted function in M. tuberculosis</th>
<th>M. leprae</th>
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</thead>
<tbody>
<tr>
<td>mmpE</td>
<td>Rv3085</td>
<td>Acridine, erythromycin, ethidium bromide, safranin O, pyronin Y resistance</td>
<td>ML3756</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>RND systems</th>
<th>Genes</th>
<th>Known or predicted function in M. tuberculosis</th>
<th>M. leprae</th>
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</thead>
<tbody>
<tr>
<td>mmpL6-6</td>
<td></td>
<td>Lipid transport</td>
<td>mmpL2 (ML2620)</td>
</tr>
<tr>
<td>mmpL7</td>
<td></td>
<td>PDIM transport</td>
<td>mmpL2 (ML0177)</td>
</tr>
<tr>
<td>mmpL8-14</td>
<td></td>
<td>lipid transport</td>
<td>mmpL2 (ML1231)</td>
</tr>
</tbody>
</table>

Ps. denotes pseudogene; del, missing probably deleted.

number compared to other fully sequenced bacterial genomes rivalled only by Pseudomonas aeruginosa.87 There are only five RND proteins predicted in M. leprae (Table 2).

In the Gram negative pathogens P. aeruginosa and Escherichia coli, RND proteins, such as MexAB or AcrAB (~1000 amino acids), act as proton motive force-dependent efflux systems and confer high levels of resistance to fluoroquinolones and other antimicrobial agents.88,89 The genetic context of the mmpL genes, encoding the M. tuberculosis RND proteins, suggested an involvement in the export of lipids or glycolipids, and a body of experimental evidence to support this has since been amassed.90,91 In particular, the MmpL7 protein is responsible for the export of the complex lipid phthiocerol-dimycocerosate (PDIM) and in M. leprae, modifies PDIM to produce phenolic glycolipid 1 (PGl1). MmpL7 may be
involved in the transport of PGL1. Given the similarities with other RND transporters it is possible that the MmpL proteins can also act in drug efflux, and as *M. leprae* produces far fewer than the tubercle bacillus does, it should be susceptible to more drugs as has been observed.

**NEW DRUG REGIMENS AND NEW LEADS FOR DRUG DISCOVERY**

Given the cost of developing new drugs, it seems certain that the pharmaceutical industry will not invest in the field of leprosy although tuberculosis may present a somewhat more lucrative market. Consequently, pharmacogenomics and high-throughput screening technologies will not be applied directly to *M. leprae* and we must look elsewhere for new leads for drug discovery. An exciting opening has emerged recently from studying the action of nitromimidazopyran derivatives on *M. tuberculosis* and a novel compound, PA824, shows great promise. In order to be active, PA824 requires the F420-dependent glucose-6-phosphate dehydrogenase encoded by *fgd* (Rv0407), and resistance mutations reside in this gene which is also conserved in *M. leprae* (ML0269). It is thus conceivable that PA824 could find use in leprosy treatment and a particularly attractive feature of this drug is its action under microaerophilic growth conditions similar to those which *M. leprae* is believed to favour.

Another area where novel drug targets may be found is in signal transduction. In most bacteria, changes in gene expression in response to environmental cues are mediated by the His-Asp phosphorelay system effected by the two-component system. These are common in prokaryotes and comprise a membrane-bound sensor protein with histidine kinase activity which phosphorylates an aspartyl residue in a response regulator protein that in turn controls the target genes. In mycobacteria, a second, eukaryotic-like phosphorelay system may be found in the form of the serine-threonine protein kinases and their cognate phosphoprotein phosphatases and these may also control cellular processes such as division and septation. The pharmaceutical industry has batteries of lead compounds for both of these protein kinase families, since there has been intensive research into the histidine kinases as novel drug targets in recent years, and numerous inhibitors of serine-threonine protein kinases have been developed for use in cancer treatment. If these kinase inhibitors became available, they should certainly be tested on *M. leprae* as this pathogen has only four complete two-component systems and four serine-threonine protein kinases, thus increasing the chances of attaining complete inhibition.

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