microarray profiling the researchers were able to identify the active antimycobacterial agent in a complex mixture. The transcriptional profiles of *M. tuberculosis* when exposed to a crude extract prepared from an invertebrate known to produce ascididemin or to the pure compound were similar. This proof-of-principle shows the power of this system to readily provide insight into drug class and likely mechanism of action—vital information, particularly for antimicrobials that are developed from high-throughput screening[13,14].

Despite being an obligate human pathogen, *M. tuberculosis* inhabits a surprising number of environmental niches within the human host, and it implements survival programs that allow it to persist or proliferate according to changing patterns of immune containment. Post-genomic technologies have begun to define these bacterial survivasomes and offer cause for optimism in the search for shortened and simplified tuberculosis drug regimens.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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The survival kit of *Mycobacterium tuberculosis*

Digby F Warner & Valerie Mizrahi

Persistence of *Mycobacterium tuberculosis* in humans depends on its ability to survive within the host macrophage. So the bacterium must resist antimicrobial mechanisms or subvert macrophage signaling pathways to prevent its death. Recent evidence suggests that the two strategies are not mutually exclusive.

Macrophages are phagocytes at the frontline of host immune defense against microbial pathogens. They are also the primary habitat of *Mycobacterium tuberculosis*: unlike bacteria that dependent on the avoidance of phagocytosis to survive, *M. tuberculosis* preferentially targets macrophage vacuoles. This apparent incongruity demands that *M. tuberculosis* either tolerate the macrophage's antimicrobial effectors—low pH and reactive oxygen and nitrogen species—or actively subvert normal cellular mechanisms to avoid being killed. Another critical feature of *M. tuberculosis* pathogenesis, extended survival in the host in a state of clinical latency, also requires similar abilities. In this case, however, *M. tuberculosis* must evade or interfere with immune surveillance and signaling pathways. Alternatively, the bacillus must tolerate host defense mechanisms, either through the mobilization of repair or detoxification pathways, or through phenotypic tolerance developed as a result of metabolic adaptation or quiescence. Three recent papers have elucidated different aspects of these strategies[1–3], influencing our current thinking on the relationship between the invading pathogen and its host, and informing an emerging theme in mycobacterial pathogenesis—that *M. tuberculosis* is a well equipped adversary that has adapted exquisitely to life as an intracellular pathogen.

Macrophages are potent producers of reactive nitrogen species, including nitric oxide (NO), in response to immunostimulatory signals. Previous mouse studies have established a critical role for NO in controlling *M. tuberculosis* infections[4]; however, NO also participates in cellular signaling pathways and respiratory inhibition. Voskuil *et al.*[1] postulated that, in addition to its known antibacterial activity, NO might be involved in bacillary persistence. Using whole-genome expression profiling, the authors investigated the transcriptional response of *M. tuberculosis* to NO. High NO concentrations elicited a response indicative of general cellular stress. By contrast, exposure of the bacterium to nontoxic NO levels or to hypoxic conditions resulted in the rapid induction of a defined set of 48 genes—the 'dormancy regulon'.

In other words, the physiological and metabolic changes associated with adaptation to hypoxia overlap with those occurring in response to low-dose NO exposure. This overlap suggested that the reversibility of bacteriostasis induced by both hypoxia and NO treatment might depend on metabolic shutdown mediated by elements of the dormancy regulon. It also suggested that oxygen and NO might competitively modulate dormancy regulon expression.

Included in the dormancy regulon are genes associated with anaerobic metabolism and stabilization of cellular components, indicating that its transcriptional upregulation might function to prepare the cell for extended periods of metabolic quiescence. Consistent with this model, Voskuil *et al.*[1] observed elevated expression of five sentinel dormancy regulon genes in mouse lungs at 21 days after infection—a time-point that coincides with the onset of immunity and bacterial growth arrest. Notably, parallel studies in mouse macrophages[5] and in the mouse *in vivo*[6] established that upregulation of dormancy genes requires the activity of inducible nitric oxide synthase. Induction of dormancy genes within the mouse phagosome seems therefore to be NO dependent[5]. On the basis of these observations, Voskuil *et al.*[1] proposed a model in which NO production and hypoxia combine to inhibit aerobic respiration and growth of *M. tuberculosis*. That is, NO production might signal the bacterium to adopt a quiescent physiological state.

Subsequent studies[7,8] have shown that the dormancy regulon is controlled by a two-component response regulator system comprising the dormancy survival regulator—DosR—and its corresponding two sensor kinases, DosS and DosT. The precise molecular signals to which the sensors respond have yet to be identified. However, recent evidence[9] of strong antibody

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responses to dormancy regulon genes in people with active tuberculosis and in latently infected individuals supports the relevance of this regulon to human disease.

Internalization of invading microbes in phagosomal vacuoles usually culminates in the fusion of the phagosome with late endosomes and lysosomes, and the destruction of the invader. *M. tuberculosis*, however, prevents phagosome-lysosome fusion, ensuring an intraphagosomal environment friendly to the bacterium. Phagosome maturation arrest is a complex process that is not fully understood: one aspect of it involves the interference of mycobacterial lipid products in intracellular trafficking events[10], but the process is likely to be multifactorial[11].

In a second key recent paper, Walburger *et al.*[2] focused on the *M. tuberculosis* proteins responsible for interfering with lysosomal delivery. They speculated that pathogenic mycobacteria (including *M. tuberculosis*) might interfere directly in host trafficking pathways through expression of their own signal transduction molecules. Specifically, they focused on protein kinase G (PknG), which is one of several eukaryotic-like serine/threonine protein kinases found in *M. tuberculosis* and related organisms. By constructing a *pknG* deletion mutant of *M. bovis* BCG, the authors showed that active secretion of the kinase within macrophage phagosomes inhibited phagosome-lysosome fusion (**Fig. 1**). The mutant had no growth abnormalities *in vitro*; however, infection of macrophages resulted in the containment of bacilli within phagosomal vacuoles displaying markers of lysosomal delivery. In contrast to the parental strain, growth of the *pknG* mutant was completely inhibited within the mature vacuoles. Furthermore, the mutant phenotype mapped to loss of the kinase activity of PknG, supporting the idea that pathogenic mycobacteria employ eukaryotic-like signal transduction mechanisms to modulate host cell trafficking pathways. Moreover, by screening a library of compounds against PknG, the authors identified a specific inhibitor of this kinase. Treatment of infected macrophages with the inhibitor resulted in the dose-dependent inhibition of bacterial growth inside macrophages, validating PknG as a potential drug target.

It is noteworthy that, in contrast to the situation in *M. bovis* BCG[12], other studies of PknG in *M. tuberculosis*[13] and the related actinomycete *Corynebacterium glutamicum*[14] have implicated this kinase in the regulation of glutamine metabolism and in the regulation of 2-oxoglutarate dehydrogenase, an enzyme of the tricarboxylic acid cycle. These findings point to probable differences in the physiological roles of PknG among organisms.

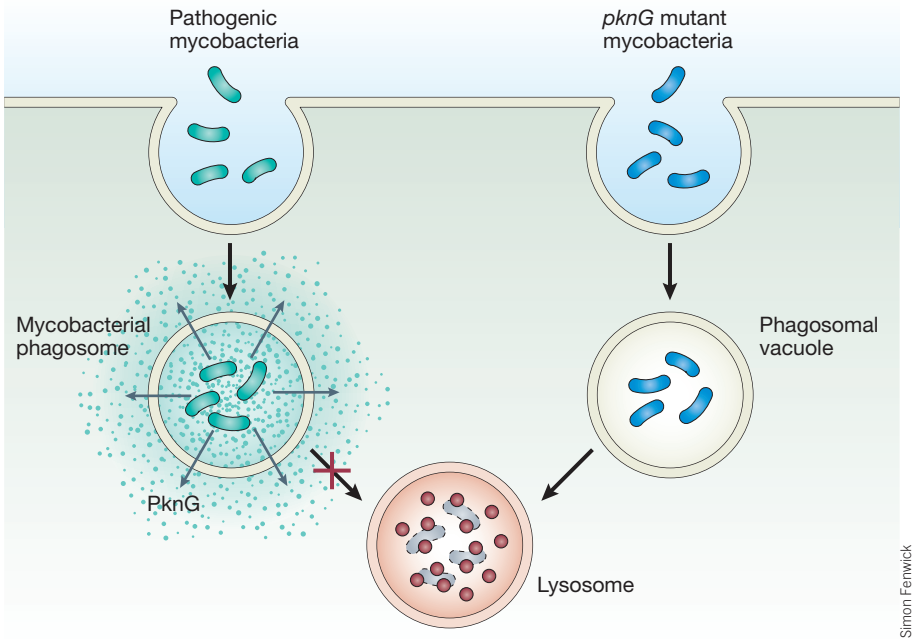

Figure 1 PknG affects the intracellular traffic of *M. tuberculosis* in macrophages. Most microbes and nonpathogenic mycobacteria quickly find themselves in lysosomes, where they are killed. By contrast, *M. tuberculosis* stays within phagosomes; the bacterium releases PknG to block phagosome-lysosome fusion. Bacteria lacking *pknG* are rapidly transferred to lysosomes and eliminated. Modified from ref. 20.

A third key study on the adaptive strategies of *M. tuberculosis* involved a genome-wide screen for *M. tuberculosis* genes required for intracellular survival[3]. The authors had previously applied a novel transposon mutagenesis method—transposon site hybridization (TraSH)—to identify genes required for optimal growth of *M. tuberculosis in vitro*[15] and for growth *in vivo* in a mouse model of infection[16]. TraSH represents an innovative modification of the signature-tagged transposon mutagenesis technique[17] in which pools of transposon mutants grown under different selective conditions are labeled with separate fluorophores and cohybridized on a microarray, enabling the identification of differential gene requirements (see related News and Views by Lamichhane and Bishai)[18]. Rengarajan and colleagues[3] used TraSH to identify *M. tuberculosis* mutants that failed to grow inside primary macrophages. By setting up three macrophage models simulating separate stages of disease, the authors identified genes required for specific stages of macrophage infection. In the first model, they used resting macrophages to simulate initial or latent infection. In the second one, macrophages were preactivated with interferon-γ before infection with *M. tuberculosis*, modeling an active immune response. In the third one, macrophages were activated with interferon-γ after infection, exploring the ability of infecting bacilli to modulate interferon-γ–mediated signaling. The authors then stratified 'survival' genes into three categories: cell-autonomous genes required for bacterial survival in macrophages and *in vivo*, genes required for *in vivo* infection but dispensable for macrophage survival, and genes important for the macrophage but dispensable for infection.

To assess the predictive value of regulated gene expression as a proxy for being required for survival of *M. tuberculosis* under the condition in question, Rengarajan *et al.*[3] compared the TraSH survival data with expression profiling data from infected macrophages[5]. In stark contrast to other bacterial pathogens, in which virulence factors are typically highly regulated, there was poor correlation between *M. tuberculosis* gene expression in macrophages and requirements for survival. For instance, many bacterial genes highly induced in macrophages and in response to NO and hypoxia (including dormancy regulon genes) were dispensable for intracellular growth. Constitutive expression of genes required for survival may therefore be an important factor in the exquisite adaptation of *M. tuberculosis* to its complex lifestyle in the human host.

The results of these and other studies[19] underscore the breadth and elegance of the tactics employed by *M. tuberculosis* to ensure its survival in the intracellular environment. The main challenge for the future will be to use the insights that have been gained in order to guide and inform the development of novel antitubercular agents with potent sterilizing activity.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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Mycobacterium tuberculosis virulence: lipids inside and out

Sabine Ehrt & Dirk Schnappinger

During infections, *Mycobacterium tuberculosis* has to acquire nutrients and resist host defense. Lipids are important for both: host-derived lipids provide food, whereas pathogen-derived lipids mediate immune suppression.

Lipids have long been thought to play key roles in the pathogenesis of *Mycobacterium tuberculosis*. In early studies, Segal and Bloch found fatty acids rather than carbohydrates to stimulate respiration of *M. tuberculosis* if the bacteria were isolated from mouse lungs instead of liquid cultures[1]. *In vivo* growth or persistence of *M. tuberculosis* were therefore hypothesized to depend on fatty acids as a major nutrient source. Forty-four years later, McKinney *et al.* described a *M. tuberculosis* mutant in which a gene encoding an isocitrate lyase, now termed *icl1*, had been inactivated[2]. In contrast to wild-type *M. tuberculosis*, this mutant showed a selective survival defect during the chronic phase of infection using a mouse of model of tuberculosis; growth during the acute phase of infection and in liquid media was unaffected.

Isocitrate lyases catalyze an essential reaction of the glyoxylate shunt, an anaplerotic pathway that bypasses the CO_2-generating steps of the tricarboxylic acid cycle and enables bacteria to synthesize carbohydrates and replenish tricarboxylic acid cycle intermediates from fatty acid–derived acetyl–coenzyme A (**Fig. 1**). For many bacteria, this pathway is required for growth on fatty acids as a sole carbon source. So the impaired persistence of the *icl1* mutant implied that *M. tuberculosis* depends on fatty acid metabolism to resist killing by an activated immune system.

Recent work by Muñoz-Elias and McKinney[3] not only confirmed these findings but also expanded previous views on the significance of isocitrate lyases in the virulence of *M. tuberculosis*. The genomes of most *M. tuberculosis* strains encode not one but two functional isocitrate lyases: ICL1 and ICL2. Muñoz-Elias and McKinney investigated the importance of these enzymes for growth on different carbon sources *in vitro*, as well as for growth and persistence in mice, using mutants with deletions in *icl1*, *icl2* or both[3]. All mutants grew like wild-type *M. tuberculosis* in media containing glycerol as carbon source, and little impact was observed on growth with glucose. Mutants containing deletions of either *icl* gene similarly grew well with most short- and long-chain fatty acids. In contrast, the *icl* double mutant (Δ*icl1*Δ*icl2*) failed to grow on fatty acids.

Studies in mice confirmed that deletion of *icl1* caused about a tenfold reduction in bacterial titers in lungs during the chronic phase of infection. Loss of *icl2* alone did not affect growth or persistence of *M. tuberculosis*. Surprisingly, *M. tuberculosis* lacking both *icl1* and *icl2* failed to grow at all in mouse lungs. Even though 1,000 colony-forming units (CFU) were detected in the lungs of mice one day after infection, less than 100 CFU were detected about 10 days later and no CFUs could be recovered after 2 or more weeks. *M. tuberculosis* devoid of all isocitrate lyase activity was therefore not only unable to persist in the face of an activated immune system, but also incapable of growing in immunocompetent and immunodeficient mice.

Although ICL1 and ICL2 of *M. tuberculosis* are also essential for a second biochemical pathway that is required for growth of *M. tuberculosis* on odd-chain fatty acids (the methylcitrate cycle)[4,5], the observed essentiality of isocitrate lyase activity for *in vivo* growth is more likely to be explained by its role in the glyoxylate cycle. This is because inactivation of enzymes of the methylcitrate cycle other than ICL1 and ICL2 do not impair growth or persistence in mice[5].

Standard tuberculosis chemotherapy requires people to take four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) for two months followed by two drugs (isoniazid and rifampicin) for four months, and it is generally effective. Unfortunately, 'generally effective' does not mean 'always effective'. Although the reasons for treatment failure vary and are always tragic, the spread of drug-resistant *M. tuberculosis* is especially alarming. Strains categorized as multidrug resistant (MDR) are resistant to at least the two first-line tuberculosis drugs (isoniazid and rifampicin), whereas those described as extensively drug resistant (XDR) are additionally resistant to fluoroquinolones and one or more of three injectable second-line drugs (capreomycin, kanamycin and amikacin). XDR tuberculosis cases have been described in at least 17 countries on all continents[6]. Enzymes such as the isocitrate lyases, which might be required for growth and survival of *M. tuberculosis* in all infected individuals, immunocompetent and immunocompromised, but are not part of human metabolism, are therefore attractive

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